European Monitoring Centre for Drugs and Drug Addiction

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COST is supported by the EU Framework Programme Horizon 2020

PROGRAMME

Testing the waters 2017

Wastewater-based epidemiology: current applications and future perspectives

3rd international conference

26–27 October 2017

Lisbon Congress Centre, Lisbon

Thursday 26 October 2017	
09.30	Registration opens
10.00	**COST Action ES1307–Sewage biomarker analysis for community health assessment: Presentation of results (Open pre-meetings)**
10.00	Overview of results WG1 – Sewage biomarkers analysis: methods and technology — Lubertus Bijlsma, Spain (WG chair)
10.45	Coffee break
11.00	Overview of results WG2 – Innovative techniques for community health assessment — Alexander van Nuijs, Belgium (WG chair)
11.30	Overview of results WG3 – Integration with epidemiology and social sciences — Malcolm Reid, Norway (WG chair)
12.00	Lunch break
	Conference opening
13.00	Opening
13.05	The role of wastewater based epidemiology for European drug monitoring: lessons learned and future challenges — Paul Griffiths, EMCDDA
	Plenary session 1 **Routine monitoring of drugs in wastewater: state of the art** Chairs: Sara Castiglioni, Italy and Erik Emke, the Netherlands
13.20	**Wastewater-based epidemiology and its practical implications in Finland — Teemu Gunnar, Finland** (keynote speaker)
13.50	The challenges of performing large-scale multi-city wastewater-based epidemiology studies — Kevin Thomas, Australia
14.10	Do environmental conditions in sewers influence drug consumption estimates in my catchment? — Christoph Ort, Switzerland
14.30	Wastewater-based epidemiology: a practical application of sewage analysis to back-calculate heroin consumption in Switzerland — Robin Udrisard, Switzerland
14.50	Methadone maintenance programs and wastewater biomarkers assessment in the city of Lisbon and major suburban regions — Álvaro Lopes, Portugal
15.10	The impact of a major music festival and tourist season on the drug, alcohol and tobacco consumption in a Croatian coastal city — Senka Terzic, Croatia
15.30	Coffee break
	Plenary session 2 **Building bridges over troubled water – combining wastewater with other data sources** Chairs: Liesbeth Vandam, EMCDDA and Pim de Voogt, the Netherlands
16.00	**Using multiple data sources including wastewater analysis to understand a local drug market — Frank Zobel, Switzerland** (keynote speaker)
16.30	The forensic side of wastewater based epidemiology — Erik Emke, the Netherlands
16.50	An ecological study into the amounts of methamphetamine in wastewater versus hospitalizations due to psychosis in a catchment area — Jørgen G. Bramness, Norway
17.10	Association between purity of seized drugs with their daily loads measured in wastewater in an Australian catchment from 2010-15 — Phong Thai, Australia
17.30	Drug use in the Austrian city Innsbruck monitored by wastewater analysis — Herbert Oberacher, Austria
17.45	Correlation of wastewater and forensic samples: Investigating the temporal use of new psychoactive substances in South Australia — Richard Bade, Australia
18.00	End of the first day
20.00	Conference dinner

Friday 27 October 2017	
	Plenary session 3 **Future perspectives: new applications of wastewater-based epidemiology** Chairs: Alexander van Nujis, Belgium and Kevin Thomas, Australia
9.00	**Application of wastewater-based epidemiology in China – from wastewater monitoring to drug control efforts — Xiqing Li, China** (keynote speaker)
9.30	Using wastewater as a tool to understand legalized retail sales effects on cannabis consumption in Washington State, US — Dan Burgard, United States
9.50	Exposure to phthalate plasticizers assessed by wastewater analysis — José Benito Quintana, Spain
10.10	A new analytical strategy to evaluate community-wide exposure to endocrine disrupting chemicals in personal care products — Luigi Lopardo, United Kingdom
10.30	Evaluating population exposure to food contaminants through wastewater-based epidemiology: pesticides and mycotoxins as pilot studies — Sara Castiglioni, Italy
10.50	Monitoring genetic population biomarkers for public health with community sewage sensors — Zhugen Yang, United Kingdom
11.10	Coffee break
11.30	Upscaling human biomonitoring – wastewater-based epidemiology to assess exposure to organophosphate flame retardants — Frederic Been, Belgium
11.50	Quantitative proteomics for molecular diagnostics of public health: the quest for biomarkers of infectious disease — Jack Rice, United Kingdom
12.10	Assessing population exposure to tobacco-specific toxicants and carcinogens using wastewater-based epidemiology — Foon Yin Lai, Belgium
12.30	Screening new psychoactive substances in urban wastewater from different European countries — Noelia Salgueiro-Gonzalez, Italy
12.50	Lunch break
13.00	**Poster session (poster prize)**
	Plenary session 4 **Addressing the key scientific issues – technical advances in wastewater-based epidemiology** Chairs: Barbara Kasprzyk-Hordern, United Kingdom, Lubertus Bijlsma, Spain and Adrian Covaci, Belgium
14.00	Six years of interlaboratory ring-test exercises for the analysis of illicit drugs in wastewater – What have we learnt? — Alexander Van Nuijs, Belgium
14.20	Spatial differences in illicit drug use in Australia's capital and regional areas; initial results from the National Wastewater Drug Monitoring Program — Ben Tscharke, Australia Anabasine and anatabine are suitable markers of tobacco smoking' — Gerber Cobus, Australia
14.50	Assessment of MDMA consumption in three European cities from the analysis of its metabolites in wastewater — Iria González-Mariño, Spain
15.10	Harnessing the Australian Census to identify population and demographic markers for wastewater-based epidemiology — Jake O'Brien, Australia
15.30	Coffee break
15.50	The use of mobile-device-based mobility patterns to determine dynamic population normalised drug loads for wastewater-based epidemiology — Josè Baz-Lomba, Norway
16.10	Degradation of alcohol and tobacco consumption biomarkers in a real sewer — Jianfa Gao, Australia
16.30	Establishing a wastewater drug analysis laboratory in the greatest metropolis of Turkey: Preliminary results from Istanbul — Mercan Selda, Turkey
16.45	Occurrence of controlled illicit drugs and new psychoactive substances in raw wastewater samples from Athens, Greece, analyzed by LC-QTOF-MS — Nikolaos Thomaidis, Greece
17.00	Best young researchers platform and poster prize awards
17.10	Conference closing

Posters	
Alberto Celma, Spain	Investigation of New Psychoactive Substances in human urine: an analytical approach for finding potential biomarkers of NPS for wastewater analysis
Ana Causanilles, the Netherlands	Wastewater-based tracing of doping use by general population and amateur athletes
Andrew Chappell, New Zealand	Wastewater analysis to determine illicit drug consumption in New Zealand
Anne Bannwarth, Switzerland	The analysis of illicit drugs in Sydney wastewater
Ben Tscharke, Australia	Wastewater analysis during a popular school-leaver festival in South Australia
Dan Burgard, United States	Revised cannabis correction factor for back-calculation: a broader picture
Erika Castrignanò, United Kingdom	Wastewater-based epidemiology as a powerful tool for helping to tackle antibiotic resistance
Ester López García, Spain	Assessment of illicit drug and alcohol use in the city of Barcelona through a wastewater-based epidemiology approach
Francesco Riva, Italy	Assess the adherence to the pharmacological therapy: a wastewater-based epidemiology approach
Frederic Been, Belgium	Methamphetamine pyrolysis byproducts in wastewater – A way of distinguishing administration routes?
Iria González-Mariño, Spain	Multi-residue determination of psychoactive pharmaceuticals, illicit drugs and related metabolites in wastewater by ultra-performance liquid chromatography-tandem mass spectrometry
Jelena Radonić, Serbia	Levels of EDCs in Danube surface water in Novi Sad, Serbia. Is there a parallel with human obesity incidence?
Jose Antonio Baz-Lomba, Norway	From cartridge to micro-plate: A high-throughput solid-phase microextraction and pre-column dilution large volume injection method for wastewater-based epidemiology
Kang Mao, China	A novel colorimetric biosensor for methamphetamine detection
Lisa Benaglia, Switzerland	Assessing the representativeness of a population equivalent: case of ammonium
Lisa Jones, Ireland	Occurrence of phthalates in Irish wastewater
Malcolm Reid, Norway and Pim de Voogt, the Netherlands	SCORE 2.0: Aqua Forensys; Safeguarding quality of wastewater analysis data for the future
Maria Jesús Andrés-Costa, Spain	Drugs of abuse in wastewater in Valencian metropolitan area (Spain)
Marie Mardal, Denmark	Metabolism of the synthetic cannabinoids AB-CHFUPYCA and 5C-AKB-48 in freshly isolated rat hepatocytes and pooled human hepatocytes analysed by UHPLC-ion mobility-qTOF
Mário Dias, Portugal	UPLC-MS/MS analysis of illicit drugs in wastewater in the city of Lisbon and Almada between 2014–16
Meena Yadav, Australia	Occurrence of illicit drugs in aqueous environment and removal efficiency of wastewater treatment plants
Natalie Sims, United Kingdom	A novel route for determining public health: analysis of oxidative stress biomarkers in wastewater
Pedram Ramin, Denmark	Modelling illicit drug fate in sewers for wastewater-based epidemiology
Peng Du, China	Trends in methamphetamine and ketamine use in major Chinese cities from 2012 to 2016
Sara Karolak, France	Estimation of the consumption of illicit drug uses in prisons and in the general population in France using wastewater analysis
Tom G. Watkinson, United Kingdom	Development of wide-field proteomics methods for water fingerprinting applied to public health
Zeqiong Xu, China	Concentration and enantiomeric profiling of ketamine and norketamine in urine, wastewater and receiving water

The challenges of performing large-scale multi-city wastewater-based epidemiology studies

Kevin Thomas[1,2], Jake O´Brien[1], Lubertus Bijlsma[3], Sara Castiglioni[4], Adrian Covaci[5], Pim de Voogt[6,7], Erik Emke[6], Felix Hernandez[3], Cobus Gerber[8], Sharon Grant[1], Barbara Kasprzyk-Hordern[9], Jochen Mueller[1], Christoph Ort[10], Malcolm Reid, Ben Tscharke[1], Alexander van Nuijs[5], Jason White[8]

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Wastewater-based epidemiology (WBE) has matured to a stage where large-scale temporal and spatial multi-city studies are being performed in Europe and Australia resulting in the collection and analysis of wastewater samples from numerous states and countries. For example, SCORE has since 2011 conducted annual week-long monitoring studies, with the number of participating cities growing from 19 in 2011 to more than 70 in 2016. Australia has commenced in 2016 the National Wastewater Drug Monitoring Programme, including 50 sites across all states and territories capturing approximately 58% of the population (14 million people). The quality of analyses is assessed through the SCORE interlaboratory study, however this is just one aspect of the WBE workflow. Being able to readily collect and analyze wastewater samples is key to bringing wastewater testing as near as possible to routine work. This requires a good relationship with wastewater treatment plants (WWTPs). Local jurisdictions or private companies often run WWTPs, and as such, certain approvals or confidentiality agreements may need to be in place before sampling can occur. An understanding of, and sensitivity to, the concerns of local authorities and WWTP owners is often necessary with solutions such as the de-identification of data sometime necessary. A good working relationship with WWTP personnel is also necessary for the collection of high quality samples and the associated data regarding the sample collection environment (such as flow data and catchment maps for obtaining population estimates). In some cases, lab personnel set up the sampling equipment and then train WWTP staff to operate it. Alternatively training and access is provided so that lab personnel may collect samples. In certain cases WWTP staff may not be willing to conduct random stratified sampling, for example, which is more difficult to plan for than collecting samples over consecutive days.

Another aspect that cannot be overlooked when conducting large-scale wastewater sampling is appropriate handling of both samples and data. Successfully shipping the samples to a lab for analysis often depends on courier companies. To ensure that samples arrive on time and in an acceptable state (ideally frozen), it is worth choosing a reputable, trustworthy courier. Furthermore, the volume of sample required, which depends on the difficulty of detecting a particular biomarker, can vary from several microliters to one liter, and as such, appropriate freezer space must be available at the analytical labs. Finally, to be able to re-analyze samples for future purposes, careful archiving of instrument data and aliquots of the original samples or sample extracts may be necessary. If archiving is not possible at a particular lab, collaboration with other labs may be required. For this reason, data management should be centralized between collaborators, with staff adequately trained in uploading quality data in a consistent format, and with dedicated staff to check data for inconsistencies.

The media has been extremely interested in the findings of wastewater analyses as well as certain proposed applications of the approach (e.g. work places and prisons). This has sometimes been to the benefit, and at

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others to the detriment, of the development of WBE and an acceptance of its potential. In some respects, WBE may also be a victim of its own success in certain quarters, with journalists, politicians and policy makers keen to apply, (mis)interpret and use the data in ways that go beyond that perceived as beneficial and possibly leading to some of the stigmatisation concerns that we have been careful to avoid and originally identified in the ethical guideline developed by Pritchard and colleagues (http://score-cost.eu/ethical-guidelines-for-wbe/). The purpose of this presentation is to share the combined experience of running large wastewater-based monitoring studies and the foreseen and unforeseen operational challenges faced.

Indicate your preference:
Platform presentation

Do environmental conditions in sewers influence drug consumption estimates in my catchment?

Christoph Ort[1], Ann-Kathrin McCall[1], Adrian Koller[1], Rocco Palmitessa[2], Frank Blumensaat[1,3] and Eberhard Morgenroth[1,3]

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Question: "How stable are drugs residues in sewers?" is a frequent question. "It depends …" is the best, answer. In this presentation we provide results from three studies to illustrate what it depends on.

Methods: 1) An innovative water quality modelling approach allowed making predictions on expected losses due to transformation for 24 substances, different catchment sizes and scenarios. The focus in this presentation is not on technical details, but on the identification of (un)stable substances, critical catchment properties and comparison of uncertainties. Some uncertainties could be reduced in theory (*e.g.*, better characterisation of transformation rates with more experiments), others not (*e.g.*, prevalence and location of consumers within a catchment). 2) In a unique full scale experiment, we quantified the transformation of 14 substances in a real sewer. 3) Environmental variables influence transformation rates in wastewater and biofilms. We constructed a novel sensor platform, the "sewer ball", to quantify relevant variables at high spatial resolution.

Results: *Modelling study*: In a medium and a large catchment (average hydraulic residence times from 4-5h and biofilm area to wastewater volume ratio of $30 m^{-1}$), <20% loss due to transformation is expected for the following frequently reported substances: benzoylecgonine, MDMA, methamphetamine and ketamine. In contrast, losses >25% are expected for cocaine, amphetamine, 6-monoacetylmorphine and 6-acetylcodeine. *Full scale experiment* (two-hour residence time, resembling a small catchment): No transformation was observed for benzoylecgonine, cocaine, MDMA and methamphetamine – as expected. 6-acetylcodeine was not detected in any sample, maybe due to small amounts discharged into the system or due to high transformation rates. Inconsistent results were obtained for ketamine (20% loss, although highly stable in previous lab experiments) and amphetamine (no transformation, despite high transformation potential in previous lab experiments), which warrants further investigations. *"Sewer ball"*: first measurements in real sewers are currently conducted and we will present results from this floatable sensor platform.

Conclusions: A water quality model helps avoiding systematic underestimation of consumption based on instable drug residues. Further research on i) transformation rates for a small number of existing and new substances and ii) spatial distribution of environmental variables is warranted to minimize uncertainty.

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If this abstract is accepted for oral presentation, it would be great if it could be scheduled for presentation **in the morning of THU 26 October, THANKS.**

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Wastewater-based epidemiology: a practical application of sewage analysis to back-calculate heroin consumption in Switzerland

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Wastewater analysis was used as a part of an interdisciplinary study about the local opioids market in the canton of Vaud, Switzerland. One of the main objectives of sewage analysis was to estimate the volume of heroin that is consumed in that region, which population size is approximately 750,000 inhabitants.

The estimate was based on samples collected over a period of nearly 3 years (from 2014 to mid-2016) in the wastewater treatment plant of the main city of the canton. Back-caculations of heroin consumption were computed from morphine loads, implying the substraction of legal morphine. The latter was estimated through all morphine deliveries in the canton over that period. Results showed that morphine loads in wastewater have increased during the monitoring period as did prescriptions of legal morphine.

Since no significant differences have been observed between week days and weekends, the mean morphine concentration of every samples was used to back-calculate the consumed heroin volume in the main city. The result was then extrapolated to the region based on the number of people receiving a substitution treatment in the catchment compared to the whole canton, assuming that this proportion also reflects the distribution of the number of heroin users.

Based on wastewater analysis, 205 kg of street purity heroin (14%) would be consumed every year in the canton of Vaud, which is a bit more than the 145 kg of a demand-based estimate. Triangulation of distinct data sources has enabled the comparison of different estimates between them and will be discussed.

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Methadone maintenance programs and wastewater biomarkers assessment in the city of Lisbon and major suburban regions

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Portugal has been participating in SCORE wastewater (WW) campaigns since 2013 and the major Sewage Treatment Plant (STP) in Lisbon have been involved in these studies. Besides the SCORE compulsory drugs and metabolites the participating consortium (NILMFS-University of Lisbon) has obtained additional data on other drugs.

Methadone and its major metabolite 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were quantified in the 2015 and 2016 week campaigns on the influents from the STP Lisbon - Alcântara.

Methadone is a long-acting μ-opioid receptor agonist that is widely used in methadone treatment programs for heroin addicts. Although supervised intake of methadone was in the past the norm for use in treating heroin addicts, now most of the medication is taken at home as self-administration. Apart the legitimate use there is always the risk of diversion for recreational use and abuse.

In Portugal, the methadone maintenance (MM) programs are supported by the General-Directorate for Intervention on Behaviours and Dependencies (SICAD) from the Ministry of Health, and the administration to the patients is insured by the Specialised Treatment Centres of the five Regional Health Administrations.

The samples were extracted by SPE and analysed by LC-MS/MS. Loads of Methadone and EDDP were determined on a basis of the inlet WW flow rates and on the population served by the STP. Methadone consumption was then estimated assuming on pharmacokinetic back calculations a metabolized fraction of methadone into EDDP as 0.55 and an excreted fraction of methadone as 0.275.

Using EDDP as biomarker, the mean weekly values found for methadone consumption (mg/day/1000 inhabitants) for 2015 and 2016 were 79.2 (SD=5.36) and 77.8 (SD=8.34), respectively.

These values were compared with data from the General-Directorate for Intervention on Behaviours and Dependencies (SICAD) for persons under MM programs covering all the 24 major urban (Lisbon) and suburban regions (Amadora and Oeiras) served by the STP. According to SICAD, the theoretical values of Methadone consumption (mg/day/1000 inhabitants) for 2015 and 2016 were 78.8 and 75.7, respectively, being quite similar to the estimations based on wastewater analysis using EDDP as biomarker. This correlation strongly suggests this last approach as a powerful tool for monitoring MM programs.

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The impact of a major music festival and tourist season on the drug, alcohol and tobacco consumption in a Croatian coastal city

Senka Terzic[1], Ivona Krizman[1], Ivan Senta[1], Petra Kostanjevečki[1], Iria González-Mariño[2], Rosario Rodil[2], José Benito Quintana[2], Marijan Ahel[1]

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In this study, we applied the wastewater-based epidemiology methodology to investigate the impact of the major music festival and tourist season on the drug, alcohol and tobacco consumption in one of the main coastal tourist areas in Croatia, the city of Split, (about 200,000 inhabitants). The study was performed in 2016 during three selected one-week periods, covering different situations: a large techno music festival (July), the main tourist season with no special events (August) and the control period outside the tourist season (November). The analyses included the biomarkers of 6 "classical" illicit drugs (cannabis, cocaine, heroin, MDMA, amphetamine and methamphetamine), 20 novel amphetamine-like psychoactive substances (NPS), 27 therapeutic opiods and their metabolites as well as the selected alcohol and nicotine metabolites. All chemical analyses were performed by LC-MS/MS, using the previously validated analytical protocols. Before the instrumental analyses, the drug biomarkers were enriched on Oasis MCX cartridges, while the metabolites of nicotine and alcohol were determined by direct injection of filtered aqueous samples into the instrument, with a previous enzymatic deconjugation in the case of nicotine metabolites.

The results indicated a prevalent consumption of "classical" illicit drugs over the investigated NPS. The mostly consumed illicit drug was cannabis (up to 15.7 g/day/1000 inhabitants), but its consumption was not clearly related to any of the investigated seasonal patterns. A rather uniform temporal consumption was observed for nicotine as well. By contrast, both investigated summer periods were characterized by significantly enhanced consumption of different psychostimulant substances, including several illicit drugs and alcohol. The most dramatic change was observed for MDMA, with a 30-fold increase in its consumption during the music festival (average daily consumption of MDMA in July, August and November were 1.8, 0.12 and 0.06 g/day/1000 inhabitants, respectively). The corresponding increase of alcohol (10-18 L/day/1000 inhabitants) and cocaine consumption (0.8-1.6 g/day/1000 inhabitants) was much lower, indicating that MDMA usage strongly predominated among the festival participants.

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The forensic side of wastewater based epidemiology

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Abstract max 350 words. Arial 11 – Justify.

The principle of using chemical analysis of wastewater to determine the use of illicit drugs was first demonstrated in 2005. Since then wastewater based epidemiology (WBE) has evolved to an acknowledged technique and widely applied. However outliers occur which cannot described to consumption only.

The Netherlands is famous for its production of Amphetamine and MDMA (XTC). The precursors needed for the production of amphetamine are controlled chemicals. Producers seek the opportunity to creatively invent new synthesis pathways to avoid the laws and invent new pre-pre-cursors which are easily transformed usually by hydrolysis to the pre-cursor. Side effect of this extra steps is the amount of chemical waste that is created during the whole synthesis process. After all the producers have to get rid of their chemical waste in a way the authorities will not notice. Here we show that fly tipping of chemical waste originating from an amphetamine synthesis in the sewer catchment area of a small sewage treatment plant resulted in failure of the treatment process.

In April 2016 at a relatively small waste water treatment plant in the Netherlands the ammonia levels were rising causing the aeration to respond automatically which was not helping to bring the levels down. Samples were measured for targeted analyses on drugs of abuse and non-targeted screening by high accurate mass spectrometry. Evidence was found for the presence of Amphetamine produced through the use of the precursor 1-Phenylpropan-2-one (BMK) by the Leuckart process through specific synthesis markers. Furthermore a range of synthesis markers were found originating form intermediate steps and a pre-precursor. This is the first time that the use of APAA as a pre-precursor for the production of amphetamine was confirmed. Furthermore a specific marker for this pre-precursor. This research shows that illegal producers of amphetamine and MDMA are not only placing containers with waste in the environment at rural areas but also discharging chemical synthesis waste directly in the sewer system. Non-target screening allowed to investigate the presence of synthesis markers in order to attribute large aberrant loads of amphetamine or MDMA to a dump of synthesis waste.

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An ecological study into the amounts of methamphetamine in waste water versus hospitalizations due to psychosis in a catchment area

Jørgen G. Bramness[1], Eline Borger Rognli[1,2], Jose Antonio Baz Lomba[3], Kevin Thomas[3], Malcolm Reid[3], Stefania Salvatore[4], Rebecca McKetin[5]

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Background: Methamphetamine is a drug of growing public health concern. The drug can induce psychosis, at least in vulnerable individuals. Earlier research has shown that there is a relationship between the incidence of methamphetamine induced psychosis and the availability of methamphetamine as measured by arrests for drug offences. The aim of the present study was to see if the use of methamphetamine in a catchment area as measured by week to week variability in waste water loads was related to admissions for drug induced and other psychosis in approximately the same catchment are.

Materials and methods: Samples were gathered by passive diffusion samples and harvested every 14 days over a 12 month period. They were investigated for 10 different drugs: methamphetamine; cocaine, and its metabolite benzoylecgonine; the metabolite of the opioid substitution drug methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP); the β-blockers atenolol and metoprolol; the antidepressant citalopram; the antiepileptic carbamazepine and the benzodiazepine oxazepam. We further were given access to data form the Norwegian Patient Register on admission to two major hospitals in the waste water catchment area; one hospital with catchment area only partially covered by the waste water plant, but the other hospital had full coverage of the waste water plant of its catchment area. We used data on admissions for schizophrenia spectrum disorder and for drug induced psychosis. Simple correlation analyses were performed.

Results: Preliminary results show that there is a positive correlation between the use of methamphetamine in the catchment area as measured by waste water concentration and the hospital admissions for both schizophrenia spectrum disorder and drug induced psychosis. This relationship was mostly present for the hospital having a good overlap between catchment area of patients and waste water, and less pronounced for the hospital with poorer overlap. The correlation was mostly present for methamphetamine and not the other drugs found in WW.

Discussion: This ecological study on the relationship between drug us in a catchment area and the incidence of psychotic disorders may point to novel uses of waste water epidemiological data.

My preference: PLATFORM PRESENTATION

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Association between purity of seized drugs with their daily loads measured in wastewater in an Australian catchment from 2010-2015

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Aims: To examine the association between the annual average purity of seized illicit drugs and their corresponding daily load measured in wastewater.

Setting/Design: Data of purity of seizure exhibits and daily loads measured in wastewater for methamphetamine, cocaine and MDMA were gathered from a catchment in Queensland for the period of 2010-2015. The wastewater data has been reported previously in Lai et al. (2016).

Catchment population: The studied sewer catchment serviced approximately 220,000 persons.

Data analysis: Statistical analysis including Pearson correlation, univariate linear regression modelling, and hierarchical regression modelling were applied to mass load and purity data to examine the association.

Findings: There was a strong linear increase in the average daily mass load of methamphetamine detected across study years (p=0.003). In the same period, the purity of methamphetamine products also increased and there was a good correlation between the annual average daily load from wastewater and the annual average of methamphetamin purity. There was a non-significant but meaningful linear increase in mass load of cocaine across study years (R^2=0.56, p=0.054), but there was no significant linear trend in cocaine purity during this time. There were no trends in mass load or purity of MDMA overthe same period.

Discussion: Although increase in actual consumption of illicit drugs depends on various factors this study demonstrates that the load increases of methamphetamine in the studied catchment could be partly attributed to changes of purity of methamphetamine in the market over the years while the purity of cocaine and MDMA did not change. Therefore, interpretation of trends in drug load estimations from wastewater monitoring need to take changes in drug purity into account.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Drug use in the Austrian city Innsbruck monitored by wastwater analysis

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Analysis of municipal wastewater for drug metabolites can reveal the scale of drug use within communities. We have applied wastewater-based drug epidemiology to get information on the drug market in the Austrian city Innsbruck.

We have developed and validated a multitarget LC-MS/MS technique for the simultaneous quantification of eighteen drugs including the stimulants amphetamine, methamphetamine, MDMA, MDA, cocaine, and benzoylecgonine. 24-hour composite wastewater samples were collected from the WWTP Rossau (Innsbrucker Kommunalbetriebe AG, Innsbruck, Austria) on four sampling periods between March 2016 and January 2017 covering 63 days. The determined analyte concentrations in wastewater were converted to estimates of community use by using available information on human metabolism, daily flow volume, population size, and dosage.

Generally, drug use In Innsbruck was found to be below European average. Among the stimulants monitored, highest concentrations and thus highest community use were observed for cocaine. Back calculation revealed that the total amount of cocaine consumed in 2016 was around 80 kg, which was unexpectedly high taking into consideration that in 2015 the overall amount of cocaine seized in Austria was 115 kg. Methamphetamine levels in wastewater were lowest among the drug monitored. This observation correlated well with reports from authorities as well as a local drug checking program.

Temporal trends revealed increasing usage rates of cocaine, amphetamine and MDMA on weekends, while opioids and other prescibed drugs were used at a consistent level through the course of the week. Lidocaine, however, represented an exception from this general rule. This compound showed a significant decrease in usage on weekends, which might be linked with its application as local anesthetic in dentistery.

Peak concentrations for cocaine, amphetamine and MDMA were observed during the festival “Bergsilvester”. For example, the daily doses of MDMA were found to be around 50-70 units on weekdays. On weekends, the mean number of daily doses increased to 175, and during “Bergsilvester” more than 500 doses of MDMA were consumed.

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ES1307 *“Sewage biomarker analysis for community health assessment”*

Correlation of Wastewater and Forensic Samples: Investigating the temporal use of New Psychoactive Substances in South Australia

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The use of new psychoactive substances (NPS) is a growing area of concern worldwide, with NPS gaining popularity, sometimes in place of more conventional illicit drugs. In Europe alone, more than 450 such compounds have been reported to date, with this number growing every year with the synthetic chemists solely limited by their ability to avoid legislation. Existing means to monitor NPS use and exposure such as self-reporting, surveys and web vendor monitoring have various limitations, with wastewater analysis being proposed as the most suitable means to provide temporal and regional trends in NPS use because it can give information on the identity and amount of drugs being used at any given time.

The vast and ever-changing scene of NPS has rendered targeted, quantitative wastewater methods undesirable, due to the time and expense involved in acquiring standards and developing methods for compounds which may not have an extended lifetime. In this regard, there has been a shift toward qualitative, suspect screening methods using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) as these do not initially require standards and the amount of compounds that can be analysed is limited only by the suspect database. However, the lower sensitivity of HRMS combined with the trace levels that NPS are found in wastewater has been detrimental in the uptake of this technique.

Our group has been analysing wastewater samples from South Australia since 2009, primarily for the “popular” illicit drugs. With a few notable exceptions, NPS were largely excluded from the method due to difficulties in selection of target compounds from the wide range of possible NPS candidates. In an effort to retrospectively show any temporal trends, the correlation of suspect screening of NPS using LC-HRMS with information garnered through forensic samples is presented. Forensic sample types include post mortem toxicology samples and drug seizures since 2013, and intelligence from interstate Forensic Facilities is also included. The correlation of forensic samples with wastewater analysis allows a more “biased” suspect screening and enabled numerous NPS to be qualitatively identified and temporal trends to be determined.

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ES1307 *“Sewage biomarker analysis for community health assessment”*

Using Wastewater as a Tool to Understand Legalized Retail Sales Effects on Cannabis Consumption in Washington State, USA

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Cannabis is the most popular illicit drug in the world with between 125 – 203 million users in the world aged 15 – 64 years using at least once in the last year.[1] In 2012 both Colorado and Washington States passed laws, legalizing the growth, production, and sale of recreational cannabis. Colorado and Washington States were called "test cases" as the rest of the country (and world) waited to see what happened as a result of these laws.[2] However, many places did not wait. In 2013, Uruguay was the first country to legalize cannabis and in the US, six more states have legalized recreational cannabis (Oregon, Alaska, California, Massachusetts, Nevada, and Maine, as well as Washington D.C.) While still federally illegal, the US Deputy Attorney General in a memo on the topic indicated that "replacing an illicit marijuana trade… with a tightly regulated market…" could help meet federal priorities.[3] Recreational sales began July 2014 in Washington, yet no data have become publically available that use direct measures to assess changes in use, public health, or the illicit trade since legalization.

This study presents the first time that wastewater-based epidemiology (WBE) has been used in the US to evaluate changes in drug consumption that are the result of a policy change. Samples were collected from the two WTPs servicing a city of 200,000 people. Samples were collected throughout the year based on day of week and quarter of year, as well as daily collections for two months each year, leading to 110 samples per year at each plant. Samples were collected for 8 months before recreational retail stores opened and for the subsequent 29 months. Samples were analyzed for carboxy-THC (THC-COOH), the metabolite of the active ingredient in cannabis. THC-COOH trends will be presented over the three year sampling campaign and compared to recreational sales data. Uncertainties exist in both the recreational potency data and in THC-COOH back-calculations; however an estimate of the current recreational market share will be presented in order to help address questions about the proportion of the total marijuana market that the retail market is serving.

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Exposure to phthalate plasticizers assessed by wastewater analysis

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Phthalates, diesters of the phthalic acid, are used worldwide as plasticizers in several plastic materials, representing ca. 50% of the plastic additives market. Human exposure to these chemicals is a major topic and several non-phthalate plasticizers are being introduced in the market, yet they continue to represent 70% of the plasticizers used worldwide. The metabolism of these substances in the human body produces the corresponding monoesters and other oxidized forms, which are excreted in urine, thus becoming perfect candidates for wastewater-based epidemiology (WBE) investigations on average population exposure to these chemicals.

Thus, we herein describe the development of an analytical method for the determination of 8 metabolites of 6 different phthalates in wastewater at the ng/L level by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Further, we investigated the stability of phthalate metabolites and their putative formation from phthalates occurring in sewage, before reaching the sampling point at the wastewater treatment plant. Results assured that neither the metabolites were significantly degraded nor formed from the parent chemicals, thus the WBE methodology can be used.

Finally, several 24-h composite influent wastewater samples were extracted and analyzed, so that metabolite loads (µg/day·inhabitant) were back-calculated. These values were converted into i) average urinary concentrations per person (µg/L), to be compared with urinary levels reported in Spain in the literature; and ii) population exposure to phthalates (µg of the corresponding phthalate/day·inhabitant), which were compared to the USA Environmental Protection Agency oral reference dose (RfD) and the European Food and Safety Administration tolerable daily intake (TDI). Calculated average exposure ranged from 10 to 714 µg /day·inhabitant (for benzylbutyl- and diethyl-phthalate, respectively), and surpassed the RfD/TDI values in some cases, so that further monitoring would be advisable. Therefore, WBE was proven to be a promisiong early-warning tool, when assessing human exposure to xenobiotics.

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A NEW ANALYTICAL STRATEGY TO EVALUATE COMMUNITY-WIDE EXPOSURE TO ENDOCRINE DISRUPTING CHEMICALS IN PERSONAL CARE PRODUCTS

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Antimicrobials and UV filters are used as additives in a broad range of personal care and consumer products such as soaps, cosmetics and disinfectants to protect against physical-chemical and biological agents. Unfortunately, some of them have been proven to have an endocrine disrupting activity, posing a threat to public health. Furthermore, due to their hydrophobic nature, chemicals in personal care products are potentially bioaccumulative. There are also concerns with regards to possible effects of antimicrobials in personal care products on the development of antimicrobial resistance. However very little is known about human exposure to these chemicals.

In this work the biotransformation of 8 UV filters (4-benzylphenol, benzophenone-1, benzophenone-2, homosalate, 4,4'-dihydroxybenzophenone, ensulizole, octocrylene, 3-benzylidene camphor) and 3 antimicrobials (4-chloro-3,5-dimethylphenol, 4-chloro-3-methylephenol, chlorothymol,) was investigated with the aim of identifying human-specific metabolites suitable as biomarkers of exposure by conducting in vitro experiments with human liver subcellular fractions, followed by in-vivo studies in pooled urine and wastewater. Analysis of samples was performed utilising high resolution mass spectrometry (HRMS) coupled with high performance liquid chromatography (HPLC). Raw data extracted from the system were processed with MetID software (Advanced Chemistry Development, Inc., ACD/Labs, UK) for prediction of metabolite structures.

As a result, for the first time, possible phases-I and II metabolites were identified and their presence in wastewater samples was observed suggesting that the impact of the exposure to antimicrobials, UV filters and many more chemicals might need to be reconsidered. Furthermore, we provide a new analytical approach based on a combination of in-vitro experiments and semi-targeted wastewater screening for future metabolism and epidemiological studies.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Evaluating population exposure to food contaminants through wastewater-based epidemiology: pesticides and mycotoxins as pilot studies

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Pesticides and mycotoxins are among the compounds of most concern for human health because of their common presence in products consumed by population and of their proved toxicity for human health. Human biomonitoring studies (HBM), i.e. the measurement of chemicals or their metabolites in human tissues or specimens, are usually adopted for assessing human exposure together with foodstuff analysis and dietary surveys. However, these tools can be cost and time consuming. Wastewater-based epidemiology (WBE) is a novel biomonitoring approach with the potential to provide direct information on human exposure to food toxicants at the population level. The aim of this study was to develop new applications of WBE for assessing human exposure to pesticides and mycotoxins by measuring specific biomarkers in raw wastewater.

The selection of biomarkers was performed by screening the available HBM studies to identify the substances most frequently detected. Specific analytical methods based on liquid chromatography-tandem mass spectrometry were developed and validated to measure the selected biomarkers in wastewater. For pesticides, the frequency of detection and abundance of metabolites were in line with the profiles reported in urine in HBM studies. Spatial differences were observed in different countries in Europe for several classes of pesticides, and seasonal variations in human intake of pyrethroids were also seen with higher intakes during spring. Pyrethroids levels were also used to back-calculate the population intake and to compare this value with the acceptable daily intake (ADI). For mycotoxins, deoxynivalenol was the substance most frequently detected in wastewater in accordance with HBM.

Results were very promising and demonstrate that measuring pesticides and mycotoxins biomarkers in raw wastewater can provide information on the collective population exposure to these substances. WBE can be therefore used as a novel complementary biomonitoring tool able to provide supplementary information to HBM studies with the advantage of giving objective and up-to-date information on the levels of exposure to food toxicants in different populations. This is particularly useful for public bodies and international agencies to guide actions and measures for policy making and evaluate policy actions aimed at reducing exposure to potentially hazardous food pollutants.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Monitoring Genetic Population Biomarkers for Public Health with Community Sewage sensros

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Wastewater-based epidemiology has recently been shown to be an innovative and promising tool for the estimation of community-wide drug use and public health. In this context, we recently proposed the use of community sewage sensors as rapid and inexpensive alternatives to classical analytical methods for the detection of sewage biomarkers for public health assessment[1]. We have demonstrated that community sewage sensors are able to detect a wide range of markers (e.g. prostate specific antigen[2], DNA[3] and illicit drugs such as cocaine[4]). Here we report a rapid "sample-to-answer" platform for the monitoring of genetic biomarkers within communities by analysis of wastewater. The assay is based on the loop-mediated isothermal amplification (LAMP) and shows for the first time the ability to rapidly quantify human-specific mitochondrial DNA (mtDNA) from raw wastewater samples. mtDNA provides a model population biomarker associated with carcinogenesis including breast, renal and gastric cancers. We integrated a membrane filter (to remove solid impurities and to perform DNA extraction and enrichment) enabling the sample to be introduced into a low cost lateral flow-based format. We demonstrated mtDNA detection over seven consecutive days, achieving a limit of detection of 4 copies of human genomic DNA per reaction (20 µl) The assay can be performed at the site of sample collection, with minimal user intervention, yielding a result within 45 mins and providing a method to monitor public health from wastewater, in the field.

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COST is supported by the EU Framework Programme Horizon 2020

Upscaling human biomonitoring – Wastewater-based epidemiology to assess exposure to organophosphate flame retardants

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Organophosphate flame retardants (OPFRs) have been increasingly used as replacements for brominated flame retardants, which have been banned due to high toxicity, persistence and bioaccumulation[1]. OPFRs are frequently used as additives in consumer products such as furniture, textiles, electronics, paints,… and are easily released to the environment. Potential health effects of certain OPFRs include carcinogenicity, neurotoxicity, allergies and endocrine disruption[2]. Due to their increased use and ubiquitous presence, there is an increasing concern for humans, which are exposed to OPFRs through dermal contact, inhalation, dust ingestion, as well as dietary intake.

At the individual level, exposure to contaminants is generally assessed through the analysis of specific biomarkers (i.e., native compound, phase I and/or phase II metabolites) in biological matrices. However, these studies are subject to various limitations: they require the collection of numerous samples from multiple individuals, can be expensive to organise, lack temporal dimension (i.e., individuals being sampled only once or, at best, over a 24 h period) and suffer from selection bias. These issues are particularly important when monitoring exposure to chemicals in the general population[2]. In this perspective, wastewater-based epidemiology (WBE) could complement human biomonitoring by gathering population-wide information about exposure to contaminants.

The objective of the present work consisted of developing an analytical method to detect and quantify biomarkers of exposure to OPFRs in wastewater. For this, a solid-phase extraction (SPE) protocol was developed to recover various metabolites of OPFRs. Subsequently, a liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and fully validated. Furthermore, we assessed the stability of the target biomarkers.

Biomarkers of exposure to OPFRs were detected and quantified in wastewater samples collected from different locations across Belgium. Temporal and geographical features were investigated to determine whether differences in exposure are present across time and locations. The results suggest that WBE can be efficiently used to monitor the exposure of the general population to contaminants of emerging concern.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Quantitative proteomics for molecular diagnostics of public health: the quest for biomarkers of infectious disease

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Abstract max 350 words. Arial 11 – Justify.

Proteomics is well established within clinical analysis with a range of biomarkers recognised by the FDA, most of these are biomarkers of cancer [1]. Analysis of prostate specific antigen (PSA) in serum is now a routine part of prostate cancer diagnosis, where it is used alongside digital examination to determine the need for invasive prostate biopsies. The use of proteomics for investigating public health has been reported [2], where the inflammation biomarker C-reactive protein (CRP) was quantified in the urine of ~8600 study participants (10% of surveyed population) using nephelometry. Whilst an excellent study it required the analysis of approximately 58,000 urine and 8600 serum samples, where as if the same study was performed using wastewater a single representative sample could have been collected for the whole population. Analysis of pharmaceuticals and drugs of abuse as part of wastewater-based epidemiology (WWBE) is becoming well established [3], however there is still scope for expansion particularly into examining the relationship between public health and disease.

We have developed a method for the analysis of proteins of disease using liquid chromatography coupled with mass spectrometry, using instruments similar to those already used for the analysis of small molecule biomarkers in WWBE [3]. To allow for this the protein biomarkers are initially digested using enzymes to form peptides, which are characteristic for their respective proteins. These characteristic peptides are then analysed using hydrophilic interaction liquid chromatography and either a triple quadrupole or quadrupole-time of flight instrument, with the dual instrument approach allowing for both biomarker quantification and for future retrospective biomarker analysis. The current analytical focus is on clinically recognised proteins of either general health, mainly inflammation, or proteins of cancer, focussing on prostate cancer. By pursuing this methodology we hope to achieve broad applicability with techniques currently used for the analysis of small molecules, and allow for easy uptake of protein analysis within the wider WWBE community.

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Assessing population exposure to tobacco-specific toxicants and carcinogens using wastewater-based epidemiology

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Background: Cigarette smoking is associated with different types of cancer due to the intake of numerous toxicants and carcinogens from mainstream smoke. Some of these compounds and their metabolites have been found in urine of tobacco users and can serve as biomarkers to assess exposure and potential risks of cancer development.

Objectives: This study aimed to (a) optimise and validate an analytical method to determine tobacco-specific toxicants and carcinogens in wastewater samples and then (b) to apply it for an evaluation of their occurrence and levels in different European communities.

Method: Raw wastewater samples were collected at the municipal wastewater treatment plants (WWTPs) in Belgium (n=16), Greece (n=8) and Switzerland (n=9) and analysed for the target compounds using the developed analytical method. The WWTPs covered the sewer catchment of about 29,000 and 36,000 people in the two Belgian cities, 4,000,000 people in the Greek city and 480,000 inhabitants in the Swiss city. Target biomarkers included anatabine (ANATA), anabasine (ANABA), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), *N*-nitrosoanabasine (NAB) and *N*-nitrosoanatabine (NAT).

Findings: Among the seven target analytes, NNK, NAT, ANATA and ANABA were detected frequently (70-100%), followed by NNN (<60%), whereas NAB and NNAL were not detected in the samples. ANATA and ANABA (the toxic alkaloids in tobacco) were determined at relatively high concentrations (approx. 20-60 and 10-30 ng/L, respectively), compared to NNN, NNK and NAT (the tobacco-specific carcinogens, approx. 1-2 ng/L). Preliminary evaluation of the results shows that the concentration ratio of carcinogens to alkaloids for the Swiss and Greek cities was similar among themselves and was lower than the two cities from Belgium.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Screening new pschycoactive substances in urban wastewater from different European countries

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The number of new psychoactive substances (NPS) distributed in the recreational drug market has rapidly increased in the last five years according to the European Early Warning System (EWS). NPS use involves mostly young people, but due to their increasing number and their interchangeable market it is difficult to estimate the real size of consumption. This creates big challenges for public health monitoring agencies and law enforcement authorities. Forensic analysis aims to monitor the use of these substances, but has some limitations to investigate the use on a large scale and the changing market.

This study tested the ability of wastewater-based epidemiology (WBE) to assess NPS use in Europe and monitor the patterns of use. A priority list of NPS was created according to the frequency of detection reported by different EWS systems in Europe (i.e. EMCDDA). This resulted in a list of 190 substances including 60 synthethic cannabinoids, 54 synthethic cathinones, 39 phenethylamines, 9 synthetic oppiods, 7 tryptamines, 6 piperidines, 4 aminorex derivatives, 4 natural NPS, 4 benzodiazepines and 3 ketamine analogues. Urban wastewater was collected from different cities all over Europe and a new screening method was developed in order to identify the selected substances. The method was based on solid phase extraction and liquid chromatography high resolution mass spectrometry (HRMS). A two step approach was used for analysis consisting of a full mass analysis allowing a first screening of NPS, and a more specific data independent acquisition (DIA) analysis to confirm the identity of "suspects". Specific criteria from the most recent guidelines (i.e. mass error < 5 ppm, isotope pattern and at least one fragment identified) were followed for NPS identification. Results were confirmed by comparison with those from the corresponding standards, if available, or with literature data (tentative identification). The presence of several NPS, especially synthetic cathinones, were confirmed while other ones could be only tentatively identified, mostly because standards were not available for confirmation.

This study employed successfully WBE to evaluate the use of several NPS on a large scale in Europe demonstrating that this approach is able to provide useful information also for NPS.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Six years of interlaboratory ring-test exercises for the analysis of illicit drugs in wastewater – What have we learnt ?

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One of the cornerstones of wastewater-based epidemiology (WBE) is the accurate quantification of the excretion products of illicit drugs in wastewater. Methods of analysis must, therefore, be fully validated before they are brought into routine use. Participation in quality control schemes is also strongly advised for laboratories carrying out such analysis. In 2011, SCORE started organising interlaboratory exercises to provide quality assurance for the reporting of WBE data.

Interlaboratory ring-test exercises were performed annually from 2011 to 2016 for the following illicit drugs and metabolites: benzoylecgonine (BE), cocaine (COC), methylenedioxymethamphetamine (MDMA), amphetamine (AMP), methamphetamine (METH), 11-nor-9-carboxytetrahydrocannabinol (THC-COOH), and 6-monoacetylmorphine (6-MAM). Different matrices containing the analytes at different concentrations were included: standard solution in methanol, spiked tap water samples and/or (spiked) wastewater. Over the period 2011-2016, 37 laboratories from 25 countries participated in the exercises. Most laboratories (81%) were located in Europe, while the other 19% were spread over different continents (North-America, Asia and Oceania). Participating laboratories were asked to analyse the samples according to their in-house validated analytical procedure.

Data analysis was performed in multiple steps: (i) a Grubbs' test was performed to identify outliers; these were excluded from the dataset and not taken into account in further evaluations; (ii) the group's mean and the group's standard deviation were calculated; (iii) z-scores were determined for each laboratory, for each sample and for each analyte based on the group's mean and standard deviation (1,2).

The results showed satisfactory accuracy for the majority of the seven analytes and generally good performance by the majority of participating laboratories. Not surprisingly, precision of results in the standard spiking solution was better than that observed in the more complex wastewater samples. Results for THC-COOH did however highlight some particular problems with this compound. Accuracy and precision were poor. Subsequent investigation has identified the most likely pre-analytical cause of this poor performance, and guidelines for the analysis of this compound have been amended accordingly.

This presentation will discuss the results of this six-years interlaboratory testing scheme, will evaluate the improvements made during the process and will formulate recommendations for future inter-laboratory exercises.

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References:

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Spatial differences in illicit drug use in Australia's capital and regional areas; initial results from the National Wastewater Drug Monitoring Program

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Abstract max 350 words. Arial 11 – Justify.

Wastewater analysis, also called wastewater-based epidemiology (WBE), has become a useful tool for measuring community drug use – both licit and illicit. Data derived from wastewater samples are objective, non-intrusive and can describe use of several substances of concern on a community level. In June 2016, the Australian Criminal Intelligence Commission launched a three year National Wastewater Drug Monitoring Program. The University of Queensland and the University of South Australia have been commissioned to conduct the project. Sampling started in August 2016 and monitors illicit drugs, pharmaceutical opioids, alcohol and tobacco consumption via wastewater analysis at sites in all Australian state capitals, as well as selected regional and urban centres to provide both spatial and temporal trends. It is currently the largest national illicit drug monitoring program based on wastewater-based epidemiology at such a high frequency. The program aims to collect up to 7 days of wastewater influent from 20 capital sites across Australia bimonthly and an additional 30 regional sites quarterly. This represents more than 50% of the Australian population. Samples are analysed using validated LC/MS-MS methods to determine concentrations of illicit drugs (methamphetamine, MDMA and cocaine), opioids (oxycodone and fentanyl), tobacco and alcohol. As has become the norm for, wastewater flow volumes, population size and pharmacokinetic data are utilised to back-calculate to population-normalised consumption estimates. Initial results have revealed significant spatial differences in consumption rates of drugs between capital city and regional sites. In general, higher consumption rates of methamphetamine, tobacco, alcohol, oxycodone and fentanyl were observed in the regional sites, whereas higher consumption of cocaine was observed in city sites. Overall, levels of methamphetamine were the highest of all measured illicit drugs across all regions and remains Australia's drug of concern. This national program will provide baseline and temporal drug-use data for public distribution. The results are intended to inform multiple research, health, education, law-enforcement and not-for-profit organisations on current drug consumption patterns which will allow triangulation across several data sources. The most up-to-date data will be presented.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Anabasine and anatabine are suitable markers of tobacco smoking

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The evidence linking tobacco use with adverse heatlth outcomes has become indisputable. Considerable portions of government health budgets are devoted to treatment and campaigns to reduce tobacco consumption.

Wastewater analysis has been applied as an important tool to measure the actual population scale use of tobacco. The approach removes the need for relying on self-report or collating sales records. In addition, unlike sales data, wastewater data will include tobacco consumed that has been illicitly imported or distributed. In many countries, illicit tobacco forms a significant portion of the total tobacco consumed. Most methods employed in wastewater analysis target the nicotine metabolites cotinine and hydroxycotinine. However, in countries such as Australia where strong measures are in place to discourage smoking, uptake of nicotine replacement products has been significant. Counselling programs and products such as nicotine patches and gums are heavily subsidised by the government. Nicotine from these products contributes to the total wastewater measurement and may result in overestimation of the number of cigarettes consumed via the usual back-calculation methods.

In our study, we monitored cotinine as an indicator of tobacco use in Adelaide, Australia, every two months over a period of 6 years. Two years ago, we identified anabasine and anatabine as alternative markers of smoking (Tscharke, et al, 2016). These alkaloids are present in tobacco leaves and tobacco smoke, but are not present in nicotine replacement products. Our results show that over time, tobacco consumption, as estimated by anabasine and anatabine, is declining at a faster rate than the decline in cotinine. The average yearly decline of anabasine and anatabine based on linear regression was approximately 20%, while annual decreases in cotinine (nicotine) consumption were less than 3%. This is in agreement with local health expert opinion, confirming that nicotine replacement uptake is substantial and may distort the true levels of smoking when nicotine metabolites are measured in wastewater.

*Ref: Tscharke B.J., White J.M. and Gerber J.P. Drug Test Anal. 2016, **8**(7), 702-7*

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Assessment of MDMA consumption in three European cities from the analysis of its metabolites in wastewater

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The application of wastewater-based epidemioloy to estimate the consumption of 3,4-methylendioxymethamphetamine (MDMA or ecstasy) from the chemical analysis of wastewater has been traditionally done by the measurement of the parent form. Nevertheless, this approach may lead to an overestimation of the abuse if the drug is directly disposed in sinks and toilets. This limitation may be overcome by i) chiral analyses, to separate the two MDMA enanantiomers and, therefore, distinguish between comsumption and discharge; and ii) performing the back-calculation from MDMA metabolites' analysis in wastewater.

The aim of this study was to investigate the suitability of a panel of MDMA metabolites (3,4-dihydroxymethamphetamine-HHMA, 4-hydroxy-3-methoxymethamphetamine-HMMA and 4-hydroxy-3-methoxyamphetamine-HMA) as biomarkers of abuse of the parent drug. They were selected in terms of their known percentage of excretion, their stability and their detectability in real wastewater. HHMA was rapidly discarded due to its rapid degradation in this matrix and its potential adsorption to glass surfaces when dissolved in ultrapure water (for standards). HMMA and HMA turned out to be, on the other hand, stable. An analytical method consisting of solid-phase extraction followed by liquid chromatography-tandem mass spectrometry was optimized and validated for their extraction, separation and detection. Several 24-h composite raw wastewater samples were collected in three European cities (Milano-Italy, Lugano-Switzerland and Porto-Portugal) and analyzed to quantify the levels of MDMA, HMMA and HMA. The concentrations obtained did not resemble the expected pharmacokinetic profile of MDMA, quite likely because pharmacokinetic studies may not reflect excretion patterns of the whole population (limited number of participants) and under real conditions of use (unreal administration doses, etc). Different back-calculation approaches were tested to estimate the consumption of MDMA: using single MDMA, HMMA and HMA loads and using the sum of all of them. Results were quite different; the use of MDMA+HMMA+HMA loads was proposed as the best option to balance the specific biases of the calculations based on single substances. Nevertheless, additional pharmacokinetic studies are urgently needed in order to get more accurate excretion rates and improve the estimates of MDMA use.

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Finding population and demographic markers in wastewater using samples collected during a population census

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Population size has been considered the largest uncertainty for wastewater-based epidemiology estimates. In an earlier study we attempted to address this uncertainty by identifying potential population markers in wastewater samples collected from 10 wastewater treatment plants during the 2011 Australian Census which allowed for accurate population counts to be determined. The potential markers (mostly pharmaceuticals and personal care products) were combined in a Bayesian inference model to estimate the daily (*de facto*) population size. This sampling approach was applied more comprehensively during the 2016 Australian Census for approximately 100 wastewater treatment plants covering approximately 70% of the population. In addition to accurate population data, the Census data also provide insight into demographics of communities such as age, ethnicity, gender, income, level of income.

Through both targeted and non-targeted analysis approaches of the wastewater influent samples and statistical interrogation, it is envisaged that both population size and demographic markers will be identified in these samples. Work has commenced on analysing these samples to allow for recalibration of the previous model and to screen for additional population and demographic markers. Census data become available from June 2017.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

The use of mobile-device-based mobility patterns to determine dynamic population normalised drug loads for wastewater-based epidemiology

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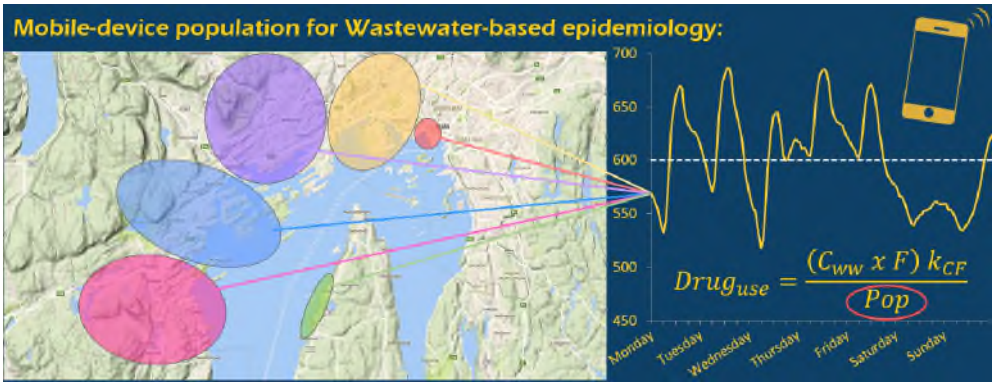
A major source of uncertainty in wastewater-based epidemiology is often the accuracy of the population data used to normalise the biomarker loads. Here we use mobile-device based population activity patterns to provide dynamic population normalized loads of illicit drugs and pharmaceuticals during a known period of high net population fluctuation.

The use of mobile-device based population activity patterns have for the first time quantified the high degree of intra-day, week and month variability within a specific wastewater catchment. Dynamic population normalization showed that per capita pharmaceutical use remained unchanged during the period when static normalization would have indicated an average reduction of up to 31%. Per capita illicit drug use increased significantly during the monitoring period, an observation that would not have been possible to measure using static normalization. The study quantitatively confirms previous assessments that population estimates can account for uncertainties of up to 55% in static normalized data. Mobile-device based population activity patterns allow for dynamic normalization that allow for much improved temporal and spatial trend analysis.



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ES1307 *"Sewage biomarker analysis for community health assessment"*

Degradation of alcohol and tobacco consumption biomarkers in a real sewer

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Abstract max 350 words. Arial 11 – Justify.

More attention is required to understand the uncertainty of sewer-based epidemiology's estimates; one of them is the stability of the biomarkers. Many stability studies have been conducted but most of them focused on the transformation of biomarkers in the liquid phase (i.e. wastewater only). More recent studies have shown in-sewer transformation of biomarkers with the presence of sewer biofilms could be significantly higher than that in wastewater due to more intensive bioactivities of the biofilm.

This study, for the first time, utilized an actual rising main (828 m x 150 mm) with detailed flow information to investigate the stability of biomarkers for alcohol and tobacco consumption. At the pump station, pumping events occurred with intervals from 30 to 150 min resulting in the sewer retention times of 2-4 hours. To better understanding the flow, acesulfame, a stable chemical, was spiked as flow tracer. Measured concentrations of hydrosulphide and methane in the rising main confirmed the strong anaerobic bioactivity in the sewer pipe. Samples that represented each 2.2 m3 wastewater pumped to the pipe was taken both upstream and downstream at each pumping event. The biomarker concentrations in each upstream-downstream pairs were measured and compared.

For alcohol biomarkers, ethyl sulfate was relatively stable with less than 25% loss while ethyl glucuronide is very unstable with more than 80% loss. For tobacco biomarkers, nicotine and hydroxy cotinine are stable. It is interesting to notice 15%-100% formation of cotinine, possibly due to the in-sewer deconjugation of glucuronide cotinine.

Ethyl sulfate could be used as a biomarker for alcohol consumption while ethyl glucuronide should not be used. In-sewer degradation of ethyl sulfate should be considered if the average retention time of the catchment is much longer than 4 hours (maximum retention time of this study).

According to the findings of this study hydroxy-cotinine is probably the best biomarker for tobacco consumption. Nicotine, cotinine, and hydroxy-cotinine would give more information about the tobacco consumption in the catchment . And in-sewer transformation of biomarkers should be considered when comparing consumption patterns of catchments with different characteristics.

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Establishing a wastewater drug analysis laboratory in the greatest metropolis of Turkey: Preliminary results from Istanbul

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Analyzing wastewater to monitor drug abuse in Istanbul and compare the obtained data with other European cities in order to identify consumption rates and drug-trafficking trends are urgent need for Turkey as well as other countries. Moreover the use of georeferenced sewage sampling systems introduces new advances for the evaluation of the obtained results considering the geographical, social and demographic aspects of the study area.

The purpose of this study is to develop a sample collection and analysis method, and apply it to two selected districts covering a population of approximately 310.000 intabitants in Istanbul. This study is carried out within the scope of a project supported by Istanbul Development Agency (Project number: TR10/16/YNY/0135).

In this study wastewater samples will be collected in two districts of Istanbul during 24 hours. After solid phase extraction and cleanup steps, sewage samples will be analyzed by LC-MS/MS method. Quantitation of cocaine, amphetamine, THC, MDMA and their urinary metabolites will be calculated. Concentrations which multiplied by daily wastewater flows and divided by the number of inhabitants (daily population normalized loads), daily consumption of parent compound based on estimated loads, back-calculations, and the associated errors will be taken into consideration for each district. Results will be presented as “µg/day/1000 inhabitant” for each compound. In order to link geographical, social and demographic aspects of the study, Geographical Information System (GIS) will also be established to interpret the results.

Although the technique still needs more optimization, error reduction effort has already been made related to sample collection, storage and back-calculation steps. This method can provide data regarding to consumed substances and/or its metabolites as well as helping to identify new psychoactive substances in the market. Moreover, GIS based studies satisfy all the ethical norms for the reason that it is performed on the mixed wastewater comes from different households, which makes all the analysis anonymous. Using GIS will assist the law enforcements to lead them to the required zones and taking necessary precautions in specific areas.

By the implementation of this project, sustainable results are intended to be collected and integrated with the SCORE group’s data after necessary requirements will be achieved.

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ES1307 *“Sewage biomarker analysis for community health assessment”*

Occurrence of controlled illicit drugs and new psychoactive substances in raw wastewater samples from Athens, Greece, analyzed by LC-QTOF-MS

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Wastewater-based epidemiology (WBE) has been applied widely for the estimation of illicit drug use *(Thomas et al. 2012; Thomaidis et al. 2016)* and it has recently been expanded to the challenging detection of new psychoactive substances (NPS) *(Borova et al, 2015; Kinyua et al. 2015)*. NPS have been introduced to the drugs market by slightly changing the structure of controlled substances in order to evade legislation.

A LC-QTOF-MS screening of controlled narcotic and psychotropic drugs and new psychoactive substances has been applied in raw wastewater samples collected in 8 consecutive days in March of four consecutive years 2014-2017 from the wastewater treatment plant of Psyttalia, Athens, Greece. A solid-phase extraction technique was used for sample preparation, using mixed bed multilayer cartridges, containing different extraction sorbents. The bbCID acquisition provided both MS and MS/MS spectra simultaneously in positive and negative ESI mode.

A database of controlled drugs and NPS was built for target screening, including precursor ions, retention time, adducts, in-source fragments and MS/MS fragments. Thresholds, such as retention time window ±0.2 min, mass accuracy ±2.5 mDa and isotopic fitting ≤200 mSigma were used as screening parameters, for the detection of the compounds. Then, a suspect list of NPS was built, using only the exact masses. For their tentative identification, specific criteria in area, intensity, signal-to-noise ratio, mass accuracy and isotopic fitting were evaluated, while the experimental retention time was compared with the predicted one from an in-house QSRR model *(Aalizadeh et al. 2016)*. For the identification of the compounds at a higher confidence level in both screening workflows, the presence of MS/MS fragments, adducts and in-source fragments was also evaluated.

This is the first wide-screening attempt of NPS in raw wastewater with a generic method and LC-HRMS. The first results indicate the occasional use of NPS in the population of Athens over the years. The target screening approach shows that specific substances, such as 2-phenethylamine and PMMA are detected every year, while MDAI, methoxetamine, MPPP are detected occasionally. From the suspect screening, N-methyl-2-AI, 3.4-MDPA, NMP were identified at a higher confidence level.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Investigation of New Psychoactive Substances in human urine: an analytical approach for finding potential biomarkers of NPS for wastewater analysis.

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New Psychoactive Substances (NPS) are constantly being developed as legal substitution of traditional drugs of abuse. Although the traditional drugs remain popular, new drugs are regularly introduced, changing the drug market ceaselessly. Monitoring NPS and its consumption is challenging as these compounds are *i)* still consumed in small quantities, *ii)* users do often not exactly know what they consume, *iii)* little information exists on metabolism of these newly introduced NPS. Therefore human biomarkers of NPS are normally missed in routine drug analysis. This highlights the need of applying a strategic workflow making use of modern analytical techniques to face this novel concern on public health safety. The workflow presented in this work consists of two stages: *1)* the collection of urine samples of individuals suspected of drug consumption and pooled urine samples from music festivals; *2)* the screening of NPS using state-of-the-art ultra-high performance liquid chromatography coupled to high resolution mass spectrometry; *3)* tentative identification of potential NPS biomarkers.

Urine samples of more than 900 individuals were taken from emergency rooms and drug addiction treatment departments from hospitals and nearly 40 samples of pooled urine were obtained from portable toilets that were located at music festivals of various music styles. The accurate-mass full-spectrum data provided by HRMS allowed the detection / identification of several NPS with high degree of reliability. The detection of these NPS in urine indicates that consumption of these compounds is a reality. However, we have mainly screened for unchanged NPS, owing to the lack of metabolic information. Thus the number of positive samples might be higher when including more NPS metabolites and performing retrospective analysis.

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Results obtained in this work may serve to select NPS biomarkers, parent NPS and/or metabolites to monitor wastewaters for estimating NPS consumption of a large community. For this purpose, sensitive targeted methodologies need to be developed to reach the low concentrations expected in wastewater.

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Wastewater-based tracing of doping use by general population and amateur athletes

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The true prevalence of doping is still unknown in elite sports, and also in amateur sports and fitness centres. Recently, analytical chemical methods have been developed that may play an important role. The analysis of untreated wastewater may reveal the excreted biomarkers of human metabolism that directly reflect the exposure to doping-related substances in the contributing community.

In the present work the potential of a wastewater-based epidemiology approach to trace undercover doping practices by amateur athletes and the general population was evaluated. Four sampling campaigns collecting 24-h composite wastewater samples were performed at the entrance of three wastewater treatment plants and one pumping station while different sport events were taking place within the catchment area. The targeted events lasted from 1 to 5 days, and the sampling period was extended to two weeks in each case to monitor the daily background use by general population and investigate whether the sport event had an impact.

In order to analyse the expected low concentrations of the chemical indicators of doping substances an analytical method based on solid phase extraction followed by liquid chromatography coupled to high resolution mass spectrometry was developed and validated. A target list of 15 illicit substances was included in the study containing anabolic steroids, weight loss products and masking agents. In addition to the target list, a suspect screening was performed to the acquired data in full scan mode to search for other metabolites and analogues.

The method was validated in terms of linearity, limits of detection and quantification, intra- and inter-day precision. Besides, the stability of the analytes in wastewater at different temperatures and matrix effects were evaluated. Preliminary results showed the presence in all samples of the use of weight loss substances. One of them, namely sibutramine, has been prohibited in the Netherlands since 2010. Besides, the presence of the anabolic steroids nandrolone and methandienone was observed with an increase in concentration in samples corresponding to the weekend. The concentrations detected during the sport events were not distinctly different from the measured daily background concentrations and therefore, no conclusion about the contribution from the amateur sport events monitored could be drawn from the current data.

This project has been carried out with the support of the World Anti-Doping Agency.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Wastewater analysis to determine illicit drug consumption in New Zealand

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Abstract max 350 words. Arial 11 – Justify.

ESR (the Institute of Environmental Science and Research Ltd) have been monitoring wastewater in New Zealand for illicit drug use since December 2016. The project started with just two sites to confirm the technique would produce useful results for Customs, Health and Police. A third site has recently been added.

Drug use in New Zealand appears to be very different to much of Europe. Similar to Australia, we have high levels of methamphetamine use, and relatively little cocaine consumption. Of particular interest, the results show significant differences between the three sites monitored in New Zealand. Not just in terms of the level of use, but also the types of drugs used.

This poster presents the results we have measured and calculated up until September 2017.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

The analysis of illicit drugs in Sydney wastewater

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Illicit drug consumption and trafficking is a major global problem. In this context, wastewater analysis offers objective and complementary information to illicit drug agencies by monitoring trends and pattern of illicit drug consumption. This aspect can be beneficial in the authorities' response to illicit drug abuse.

Firstly, the study was performed to investigate the geographical and temporal distribution of illicit drugs in Sydney and to identify possible hot spots. Secondly, possible similarities/differences in consumption between this study, another Australian study and one conducted in Switzerland by different researchers were explored.

Wastewater specimens located in four sewage treatment plants in the area of Sydney were collected in March 2016. Thirteen selected illicit drug target compounds were extracted and subsequently analysed using instrumental analysis. The amounts of illicit drug compounds measured in the wastewater were used for the estimation of the consumption. Temporal and geographical analyses were conducted to obtain a better understanding of the type and amount of illicit drugs consumed in Sydney.

Trends and geographical patterns in illicit drug consumption in Sydney were observed. The analysis of Sydney wastewater revealed that methamphetamine was consumed the most, followed by cocaine, 3,4-methylenedioxyamphetamine (MDMA) and morphine. Weekly patterns were also observed. The geographical analysis showed a higher amount of illicit drugs in the Eastern part of Sydney. This could be because this region is very demanded by young adults, surfers, tourists, and many wealthy people are settled there. The comparison of our study to another Australian study showed a high consumption of methamphetamine and MDMA in Sydney in comparison to diverse areas investigated in the different States and Territories. The comparison between Sydney and Switzerland revealed a different consumption in these countries, in line with the indicators of the traditional market.

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The authors suggest that the information obtained through wastewater analysis can be of great importance for law enforcement agencies as it provides a complimentary and objective estimation of the illicit drug consumption and by extension information about the size and evolution of the related illicit drug market.

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Wastewater analysis during a popular school-leaver festival in South Australia

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Abstract max 350 words. Arial 11 – Justify.

Wastewater analysis is valuable for describing levels of drug-taking and observing changes in use over time. Smaller areas and sub-populations can be analysed and dissected to determine whether factors such as the scale of drug use in the community, socio-economic status, events, or other demographic characteristics may play a role in drug taking or other behaviours. It has been argued that drug use in younger sub-populations has become normalised to some extent in Australia, including higher rates of use among attendees of some types of festivals. To evaluate the extent of drug use during a school –leaver festival, wastewater from a host city in South Australia was analysed before, during and after the annual events in 2013 and 2015. Drugs that were assessed included typical 'recreational drugs' 3,4-methylenedioxymethamphetamine (MDMA) and cocaine, as well as other stimulants including methamphetamine and seven New Psychoactive Substances (NPS), six opioids, nicotine and cannabis.

Results showed that MDMA was consumed in larger amounts during the festival than the weeks before or after for the 2015 festival ($P < 0.01$, $P < 0.05$, respectively), but was not significantly different between weekends for the event 2013. Consumption of cocaine, methamphetamine, all opioids, nicotine and cannabis was not significantly different before, during or after the event in either year. Some NPS, such as α-pyrrolidinopentiophenone (alpha-PVP) and ethylone, were consumed in higher amounts during the event weekend ($P < 0.01$) but levels were relatively insignificant in comparison to the scale of consumption of the other stimulants. Rates of drug-use during the festival did not seem to reflect the prevalence reported by surveys of South Australians within the same age group. Despite the few arrests that typically occur during the annual festival, widespread illicit drug consumption was not at alarming levels as described by the media.

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Revised Cannabis Correction Factor for Back-Calculation: A broader picture

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Wastewater-based approaches have been used to successfully monitor use of many illicit drugs in Europe as well as globally. This approach is used to determine levels of drugs or their metabolites as well as make back-calculations to estimate parent drug consumption. In order to perform back-calculations pharmacokinetic data is needed to understand excretion factors of the drug target residue (DTR) to wastewater. Estimates for drugs such as cocaine, amphetamine, methamphetamine, and MDMA from wastewater are robust due to the stability of the DTR in wastewater, the fraction of the DTR that stays in the aqueous fraction from urine to wastewater, and the known excretion profile of the drug. However, for other drugs the back-calculation is much more challenging. For example, the cannabis DTR excretion factors are poorly known due to differing routes of administration and fractions of the DTR in urine and feces.[1,2] Recently a refined correction factor using the DTR in urine for smoked cannabis was presented for wastewater estimates.[3] This estimate does not account for the amount of DTR excreted in feces that partition into the aqueous phase. Additionally in US markets, smoked cannabis factors may not properly represent other forms such as edibles, drinkables, and extracts for inhalation. In Washington State, these alternative forms of cannabis consumption currently make up 30% of the sales and are continuing to rise.

This study attempts to better understand the issues with cannabis excretion to provide better estimates for back-calculations used in wastewater-based approaches. This poster will present preliminary findings on the excretion factors from 10 volunteers who self-identify as cannabis users. These volunteers have consumed a known amount of cannabis with known potency from a variety of consumption routes (smoked, edible, inhaled extracts) which were purchased from the legal retail market in Washington State. Volunteers then collected their urine and feces for four days after consumption. Results for the amount of cannabis active ingredient (THC) and its metabolites (THC-OH, and THC-COOH) will be presented for both the feces and the urine. From these values, a more representative back-calculation factor will be provided.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Wastewater-based epidemiology as a powerful tool for helping to tackle antibiotic resistance

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The environmental framework is one of the key areas for tackling antibiotic resistance at global level (Berendonk, Manaia et al. 2015). This aims at reducing the amount of antibiotics (ABs) released in the environment through three main routes: animal, manufacturing and human wastes (O'Neill 2016). It is believed that an extensive (mis)use of some ABs causes antimicrobial resistance spread. It is therefore of paramount importance to undertake (near) real-time monitoring of ABs levels in the environment with an aim to verify unnecessary (excessive) use by humans and in veterinary medicine. So far, wastewater-based epidemiology (WBE) has shown its potential in near real time analysis of illicit drug (Thomas, Bijlsma et al. 2012, Ort, van Nuijs et al. 2014), alcohol (Reid, Langford et al. 2011) and tobacco use (Castiglioni, Senta et al. 2014).

This study aimed to identify and evaluate metabolic biomarkers of public exposure to ABs using the WBE concept. ABs studied were: ciprofloxacin, desethylene-ciprofloxacin, ofloxacin, norfloxacin, ofloxacin-*N*-oxide, desmethyl-ofloxacin, nalidixic acid, lomefloxacin, moxifloxacin, moxifloxacin-*N*-sulphate, prulifloxacin, ulifloxacin, ketoconazole (the only antifungal), flumequine, nadifloxacin and besifloxacin.

Hence, an analytical method, based on solid-phase extraction and liquid chromatography coupled with tandem mass spectrometry analysis, was developed and fully validated for the study of the occurrence of 16 ABs and their metabolites in wastewater. A stability study of the targeted biomarkers was also performed for 24 hours in dark biotic conditions.

The developed methodology was applied to composite samples collected over a week from a several WWTPs covering 362 km^2 and 75% of population in the South West of the UK, where many targeted antimicrobials and their metabolites were found at quantifiable levels. The most commonly found ABs were ciprofloxacin, desethylene-ciprofloxacin, ofloxacin and its metabolites, nalidixic acid and the antifungal ketoconazole.

Spatial and temporal analysis of community-wide daily consumption was undertaken. The results were compared with official statistics on prescription data. The study provided, for the first time, a comprehensive evaluation of ABs loads and consumption patterns in a large catchment study of 1.14 million people.

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Assessment of illicit drug and alcohol use in the city of Barcelona through a wastewater-based epidemiology approach

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Wastewater has revealed as a source of information on lifestyle and human activities. The organic chemicals in it reflect the food that people eat, the materials they use and even the drugs they consume. Thus, the concentrations of a specific drug or a stable drug metabolite in wastewater can be used to back-calculate the use of that drug in the population served by the wastewater treatment plant (WWTP). This approach is known as wastewater-based epidemiology (WBE), and it is an accepted tool to investigate trends and changes in drug abuse in a specific location.

A WBE approach was used to investigate illicit drug and alcohol consumption in the city of Barcelona. Annual and weekly use trends were evaluated by monitoring drug use biomarkers at the inlet of one of the main WWTPs of the city every day during one week between 2011 (2013 in the case of alcohol) and 2015. Biomarker concentrations were measured by means of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). To back-calculate drug use, daily average concentrations (from 24-h integrated wastewater samples) were normalized by the daily average water flow at the inlet of the WWTP, the people served by the WWTP (annual municipal census), and a refined correction factor that takes into account the average excretion rate of the biomarker and the molar ratio between the biomarker and the drug.

In the light of the results, and in agreement with official estimates at national level, alcohol (18 mL (14g)/day/inhabitant >15 years on average) and cannabis (38 g/day/1000 inh. aging 15-64 on average) were the most consumed drugs followed by cocaine (2.4 g/day/1000 inh. aging 15-64 on average), amphetamine-like compounds (ephedrine: 1.2 g/day/1000 inh, methamphetamine: 0.28 g/day/1000 inh., MDMA: 0.19 g/day/1000 inh., and amphetamine 0.09 g/day/1000 inh. aging 15-64 on average), and methadone (0.16 g/day/1000 inh. aging 15-64 on average). Drug use increased over the five years monitored (in agreement with official regional drug use records), except in the case of heroin and diazepam. Alcohol, cocaine, and MDMA were the only drugs showing a higher consumption during the weekend compared to the weekdays.

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COST is supported by the EU Framework Programme Horizon 2020

ES1307 *"Sewage biomarker analysis for community health assessment"*

Ort, C., A. L. van Nuijs, J. D. Berset, L. Bijlsma, S. Castiglioni, A. Covaci, P. de Voogt, E. Emke, D. Fatta-Kassinos, P. Griffiths, F. Hernandez, I. Gonzalez-Marino, R. Grabic, B. Kasprzyk-Hordern, N. Mastroianni, A. Meierjohann, T. Nefau, M. Ostman, Y. Pico, I. Racamonde, M. Reid, J. Slobodnik, S. Terzic, N. Thomaidis and K. V. Thomas (2014). "Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis." Addiction **109**(8): 1338-1352.
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Assess the adherence to the pharmacological therapy: a wastewater-based epidemiology approach

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The scarce adherence to the pharmacological treatment is one of the main causes of non-effectiveness of a therapy and this may lead to increased morbidity and worsened economic burden. This study is one of the first attempts to use watewater-based epidemiology (WBE) to face this problem, in order to provide a tool that can, in a short-term period, assess the overall adherence of a population to the medical treatment. The WBE approach was used to select and measure a panel of pharmaceuticals and specific urinary metabolites (biomarkers) and to back calculate the amounts consumed by a population. The consumption estimates will be compared with the prescriptions obtained from the official report on drug use of the Italian Medicines Agency (AIFA).

Pharmaceuticals were selected according to specific criteria to meet the requirements of a WBE biomarker. The first step was to select pharmaceuticals sold in high amounts and only under medical prescriptions in order to be able to measure them in wastewater, and to obtain a reliable estimation of the sales volumes. The most updated data from the last official report on drug use in Italy (AIFA) were considered for selecting pharmaceuticals. The second step was to include pharmaceuticals excreted both as parent compound and at least one main metabolite, which must be exclusive of the pharmaceutical considered and not used as an active principle itself. This allowed us to exclude substances which might have other sources than human metabolic excretion. Finally we included only pharmaceuticals which are administered chronically, in order to avoid seasonal fluctuations.

The pharmaceuticals and metabolites selected belong to different therapeutic classes: cardiovascular drugs (enalapril, ramipril and losartan), antidiabetic drugs (gliclazide) and antidepressive drugs (citalopram). A multiresidue analytical method based on HPLC-MS/MS was developed and validated in raw wastewater. The method was then applied for a preliminary analysis of wastewater samples collected from some wastewater treatment plants in Italy. This will allow to estimate pharmaceuticals consumption in different populations and to compare results with the sales data in order to evaluate the reliability of this new WBE approch.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Methamphetamine pyrolysis byproducts in wastewater – A way of distinguishing administration routes?

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Consumption of methamphetamine (MA) in Australia is a widespread phenomenon, yet, depending on the indicator considered, contrasting figures are obtained regarding its magnitude. Wastewater analyses show a dramatic increase in its use[1,2], whilst household surveys suggest constant prevalence rates[3]. Nonetheless, forensic and survey data indicate that consumption of high purity crystalline MA is increasing, which raises concerns due to its consumption via inhalation[4], a more problematic administration route compared to other non-injecting routes[5,6].

Smoking of MA generates various pyrolysis byproducts[7]. N,N-dimethylamphetamine (DMA), in particular, has been found in the urine of users[8], as well as in combustion residues recovered from smoking devices[9]. Whilst DMA can be found as an impurity in MA seizures, forensic analyses indicate that its abundancy relative to MA is low (approximately 0.05%). Hence, DMA and DMA/MA ratios could be used as markers of MA smoking.

This work focused on the analysis of MA pyrolysis byproducts in wastewater samples and on the detection of potential geographical and temporal differences in consumption habits in Australian communities. Solid-phase extraction (SPE) followed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) were used for quantitative analysis and suspect screening.

DMA was detected in most samples and concentrations ranged from <LOQ (i.e., 0.5 ng/L) to 39 ng/L. Significant differences (p-value < α = 0.05) in DMA/MA ratios were observed between locations. Overall, DMA/MA ratios and population normalised MA loads were positively correlated (Pearson's correlation=0.7, p<0.0001). Yet, some locations showed lower DMA/MA ratios than expected, suggesting that high MA consumption was not always associated with high prevalence of smoking. Temporal data for one location showed a decrease in DMA/MA ratios in the period 2014-2016 compared to 2011-2013. At the same time, a steep increase in MA loads in wastewater was observed. These could be due to an increase in MA purity, hence containing fewer synthesis byproducts, including DMA.

Although the interpretation of DMA/MA ratios requires information about the purity of MA seizures, which can be obtained from forensic laboratories, it can be used to identify areas with potentially higher prevalence of problematic use (i.e., smoking) and monitor the evolution over time.

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Multi-residue determination of psychoactive pharmaceuticals, illicit drugs and related metabolites in wastewater by ultra-performance liquid chromatography-tandem mass spectrometry

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Psychoactive licit and illicit drugs and their metabolites are widely known to be present in urban wastewaters due to their high rates of production and consumption. This fact may entail environmental consequences (if these residues end up in surface waters) but also act as indicator of their use in different communities, hence the importance of quantifying their levels. Traditionally, analytical methods for the determination of drugs in wastewater involve a sample pre-concentration step (usually solid-phase extraction) followed by a high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) analysis. However, the use of ultra-high performance liquid chromatography (UHPLC) is nowadays preferred over conventional HPLC due to its higher separation efficiency, what results in reduced analysis times and higher sensitivity and peak resolution.

In this line, this study presents a new UHPLC-MS/MS methodology for the determination of 39 psychoactive drug residues (including parent forms and metabolites) in raw wastewater. Analytes were solid-phase extracted on mixed-mode (reverse-phase plus cation-exchange) sorbents and further separated and detected following a rapid UHPLC-MS/MS method. The analysis of 24-h composite raw wastewater samples collected in Santiago de Compostela during one week in 2016 revealed the ubiquity of some of the analytes. The antidepressant venlafaxine, its metabolite O-desmethylvenlafaxine and norsertraline (metabolite of sertraline) were the most abundant species, with levels even >500 ng/L. Some of the targeted benzodiazepines were also detected in most of the samples, but at lower concentrations (~100 ng/L) matching their lower range of mass doses prescribed. Finally, benzoylecgonine and cocaine were the two illicit drug-related substances found at higher concentrations.

The application of the wastewater-based epidemiology principles allowed for the estimation of consumption of four benzodiazepines, four antidepressants, methylphenidate, and three illicit drugs. The most suitable biomarker of consumption was defined in every case. Refined correction factors were proposed when needed and used to assess parent drug consumption loads. Finally, average consumption figures for pharmaceuticals were converted into total Defined Daily Doses and compared with the prescription data got from the Galician Health Service.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Levels of EDCs in Danube surface water in Novi Sad, Serbia. Is there a parallel with human obesity incidence?

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Endocrine disrupting chemicals (EDCs) was found to increase the obesity and the insulin resistance that are the risk factors for type 2 diabetes mellitus. More than half (58.5%) of adult population in the north Serbian province, Vojvodina, is obese or overweight (Medic Stojanoska et al., 2016). Death rates from consequences of diabetes were almost equal in men and women (about 24.0 per 100,000) and places Serbia among the regions with the highest diabetes mortality rates in Europe (Ilic & Ilic, 2017).

The municipality of Novi Sad, the capital of Vojvodina, challenges a specific problem of getting the drinking water. The Water supply system in Novi Sad uses the ground water from alluvial aquifers, which originates partly from the Danube (80-90%) and from the backland groundwater (10-20%) (Milic et al., 2014). The main drinking water abstraction point is located only several hundred meters downstream of the two main municipal wastewater discharge sites. Other two drinking water abstraction points are located in densely populated urban areas without sewage system, and one of them is placed near the oil refinery. All the points of abstraction are also threatened with nearby extensive agricultural activities. In addition, the municipal wastewater is discharged directly to the Danube without any treatment!

Within our study, wastewater, Danube surface water and abstracted water were analyzed in order to determine the concentration levels of selected EDCs. The analysis was performed using the GC/MS technique. The human health risk was assessed using the hazard quotient and the margin of exposure methods.

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From cartridge to micro-plate: A high-throughput solid-phase microextraction and pre-column dilution large volume injection method for wastewater-based epidemiology

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The detection and quantification of illicit drug and pharmaceutical residues in sewage has been shown to be a valuable tool that complements existing approaches in monitoring the patterns and trends of drug use. However, sample preparation remains one of the principal bottlenecks in modern high-throughput analysis by liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Most of the drug target residues are found in wastewater in the ng L^{-1} range and therefore a pre-concentration step is usually required. Solid phase extraction (SPE) is the most common procedure for this purpose and large volumes (between 50 and 1000 mL) of wastewater are necessary in order to reach the required limits of. Miniaturization of the sample preparation has become an alternative in modern high-throughput methods. The proposed methodology is based on the micro-extraction of small volumes (1 mL) of wastewater using an HLB 96-well microplate and both large volume injection (LVI) and at-column dilution (ACD).

The aim of this study was to develop, validate and apply a novel high-throughput WBE procedure for the analysis of 13 drug target residues by µSPE-ACD-LVI-UPLC-MSMS. Thus, this procedure will potentially improve the technical and environmental WBE feasibility by: i) allowing for reduced sample-volumes which has a direct impact on the logistics of collecting, transporting and storing the wastewater samples ii) reducing sample preparation and analysis times, iii) decreasing the volumes of solvents used, and iv) improving method performance as measured by accuracy and precision.

The method limits of quantification were between 0.3 and 6.3 ng L^{-1}. The repeatability calculated as the relative standard deviation of six wastewater samples spiked at 200 ng L^{-1} were between 3.4 and 14.4 % while the relative recoveries for the 13 drug target residues were between 92 and 110%. The proposed workflow was successfully validated and applied to a set of wastewater samples collected in Oslo in 2016.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

A Novel Colorimetric Biosensor for Methamphetamine Detection

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Methamphetamine (METH), the second most widely-abused drug in the world, may cause severe societal consequences, such as loss of lives and health of abusers, increased treatment costs and higher incidence of crimes [1-2]. Conventional methods for the detection of METH such as gas chromatography or high performance liquid chromatography-mass spectrometry are highly sensitive and selective. However, these techniques usually require expensive instruments and tedious sample processing. As an alternative, biosensing has a great potential for rapid and on-site detection of analytes in body fluids and environment samples, due to ease for miniaturization and capability of measuring complex matrices with minimal sample preparation[3]. A biosensor is a small device with a biological receptor that generates a signal (electrochemical, optical, nanomechanical, mass sensitive, etc.) in the presence of an analyte. Here, we present a cost-effective and label-free colorimetric biosensor based on non-aggregation Au@Ag core-shell nanoparticles, for the first time, to detect methamphetamine (METH). The biosensor consisted of a reporter probe (RP) that is a specific single-stranded DNA (ssDNA) sequences coated with Au@Ag, a capture probe (CP) conjugated with superparamagnetic magnetic beads (MBs), and an illicit drug-binding DNA aptamer (Apt). The DNA aptamer could hybridize with both RP and CP, generating Au@Ag-dsDNA -MBs sandwich structure. In the presence of the METH, the sandwich structure cannot be formed due to higher affinity of the aptamer to illicit drug than that to complementary ssDNA. The concentration of Au@Ag reduces, leading to the color change the in supernatant if an external magnetic field is introduced to absorb Au@Ag-DNA-MBs. Under optimal experimental condition, our biosensors is able to detect as low as 0.1 nM (14.9 ng L^{-1}), spanning a linear range of logarithm concentration of 0.5-200 nM for METH. Other common illicit drugs had little interference on the detection of METH. Recoveries of METH in the spiked urine samples were more than 83 %. These results demonstrated that our colorimetric sensor has a significant potential to be implemented as a cost-effective and visual sensing platform to detect METH in environmental matrices.

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Assessing the representativeness of a population equivalent: case of ammonium

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Wastewater analysis was performed during a music festival in Switzerland. The festival's wastewater is conveyed to the sewage treatment plant (STP) of the city where the event takes place. This STP normally serves about 25'000 inhabitants. The festival is a regional must-attend event that attracts about 50'000 people per day. Only 5% of the festival-goers sleep at the festival campsite. This means that the inhabitants of the city and 5% of the festival-goers, that is to say the "resident" population, contribute to the STP wastewater for 24 hours. The rest of the festival population, or "transient" population, which is in large majority over the "resident" population, contributes daily to the STP wastewater for about 8 hours, that is the average time for attending the festival.

In order to estimate the size of the population contributing to the festival's wastewater, i.e. the "resident" + "transient" populations, ammonium was analysed. A population equivalent (PE) of this biomarker, representative of this specific catchment, is thus needed. However, a PE is determined over a 24-h period. During the festival, because the proportion of the population which contributes mostly to the sewage does not do it over 24 hours, but over a much shorter duration, a daily PE can not be used. Indeed, it would underestimate the size of the population contributing to the wastewater during the festival. It is therefore necessary to find an alternative.

To do this, we propose two approaches. The first one uses the festival's statistics coupled with census data of the city. The second approach adapts the PE of ammonium to the 8-h period which is then applied to the estimated proportion of ammonium loads coming from the "transient" population. The first approach provides a relatively stable daily population size, while the second one shows values that can vary by a factor of two, due to ammonium loads variations, which are themselves caused by flow variations. Since no approach is ideal, their respective limitations will be discussed. The impact of these results on per capita loads of the target illicit drugs will be presented.

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Occurrence of phthalates in Irish wastewater

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Phthalate esters are a group of synthetic organic chemicals that are most commonly used as plasticizers in polyvinyl chloride (PVC) materials. Common end uses for PVC, and therefore phthalates, include tubing, blood bags, medical devices, clothing, flooring, packaging, toys, automobile parts, flooring, and roofing. As phthalates are so commonly used, their impact on the environment and human health has been extensively studied. Phthalates have been found to be recalcitrant, ubiquitous within the environment, and in many cases, detrimental to human and animal health. This project represents an important collaboration between three research centres (DCU, ASU, & NIVA) with support from Irish Water, Panda and Fingal Co. Co., to assess the potential sources and environmental fates of priority phthalates in Ireland. The impact of such study would be the analysis of these eleven phthalates from source to fate, in order to inform environmental policy on the risks posed by phthalate usage.

The phthalates investigated were Benzylbutylphthalate (BBP), Dibutylphthalate (DBP), Dipentylphthalate (DPP), Diisopentylphthalate (DIPP), Diethylhexylphthalate (DEHP), Dihexylphthalate (DHP), Diisobutylphthalate (DIBP), Di-n-octylphthalate (DNOP), Diisononylphthalate (DINP), Diisodecylphthalate (DIDP) and Dimethylphthalate (DMP). Samples were collected from 3 Irish WWTPs and analysed by GCMS and LC-MS/MS.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Drugs of abuse in wastewater in Valencian metropolitan area (Spain)

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Overall, pollution by drugs of abuse at very low concentrations appears to be broad in environmental compartments near populated areas. This situation could bring about potential risks for human health and ecosystem. There are several direct and indirect pathways through which drugs of abuse can be introduced into the aqueous environment [1,2]. Insufficiently treated municipal wastewater discharge is the major route responsible for surface water contamination [3]. In addition, back-calculation from the concentration of illicit drug in the influents of wastewater treatment plants (WWTPs) provides an important tool for estimating its local consumption.

In this study, 42 drugs of abuse including traditional and emerging ones were analysed in influent and effluent of 3 WWTPs. A reliable method using solid-phase extraction and ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) achieved determination of up to 42 drugs of abuse. Samples (24 h composite) were collected at the WWTPs of Quart-Benàger and Pinedo I and II during 5 consecutive years. These plants treat most of the residual water from Valencia City and its metropolitan area.

The determination of drugs of abuse in the influent of the selected WWTPs shows the presence of methadone and morphine (at concentrations up to μg L^{-1}), while heroin was not detected. Regarding amphetamine-type stimulants, amphetamine, methamphetamine, ethylamphetamine, methylphenidate, ephedrine and bufotenine were in most influent samples at concentrations of ng L^{-1}. Cocaine and its metabolites benzoylecgonine, cocaethylene and ecgonine methyl ester were also at concentrations ranged between μg L^{-1} and ng L^{-1}. Cannabinoids and other drugs of abuse as ketamine and 4-methoxyphencyclidine were only detected in influent samples at concentrations of ng L^{-1}.

The effluent samples analyzed show that all compounds were removed at all except ephedrine, benzoylecgonine, 3,4-Methylenedioxymethamphetamine (known as Ecstasy), ketamine, morphine and methadone at concentrations lower than ones detected in influent samples.

Furthermore, the consumption of selected drugs of abuse was estimated for the population in Valencian metropolitan area. The most consumed drugs of abuse were cocaine and cannabis followed by amphetamine-type stimulants. The temporal variation of these drugs shows little variation from one year to other showing a quite stable consumption.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Metabolism of the synthetic cannabinoids AB-CHFUPYCA and 5C-AKB-48 in freshly isolated rat hepatocytes and pooled human hepatocytes analyzed by UHPLC-ion mobility-qTOF

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Analytical strategies for detection of drugs in wastewater rely on information on the metabolism and excretion. Such data can be sparse or limited for new psychoactive substances (NPS). The synthetic cannabinoids is a group of NPSs known to be extensively metabolized in man and the main urinary targets are the metabolites. The liver is the main metabolic organ in human, incubation of NPS with (pooled) hepatocytes can thus form a basis for analytical target identification.

The aims of the study were to identify main human urinary metabolites of the synthetic cannabinoids 5C-APINACA and AB-CHFUPYCA using pooled human hepatocytes (pHH) and freshly isolated rat hepatocytes (fiRH). The presented results will form the basis for a discussion on *in vitro* methods for the identification of major analytical targets excreted in both urine and feces to be used in WBE.

5C-APINACA and AB-CHFUPYCA were incubated for 3 hours at concentration levels of 10 µM with fiRH or pHH in Krebbs-Hanseleit buffer. The viability of the hepatocytes were above 95 %. Samples were precipitated with methanolic internal standard solution (5F-APP-PICA). Ten µL was injected onto ultra-high performance liquid chromatography - ion mobility – quadrupole time-of-flight mass spectrometry (UHPLC-IM-qTOF) in MS^E-mode, which was controlled by UNIFI 1.8.2 (Waters, Manchester, UK). The metabolite identification was supported by *in-silico* metabolite generation software (Meteor, Lhasa ltd), and UNIFI *in-silico* fragmentation tools.

The main metabolic pathways for 5C-APINACA were hydroxylation(s) on the adamantyl-moiety with subsequent glucuronidation as well as oxidative dehalogenation which is previously reported for the ω-fluoro-type synthetic cannabinoids. The main metabolic pathways for AB-CHFUPYCA were the hydroxylation(s) of the cyclohexane group, as well as oxidative deamination. The ion mobility cell ensured cleaner mass spectra of the synthetic cannabinoids and their metabolites, which eased the assignment of fragment ions to precursor ions. Based on these findings, the main urinary analytical targets for 5C-APINACA should be ω-COOH-APINACA together with verification of HO-adamantyl-5C-APINACA to distinguish from consumption of APINACA and 5F-APINACA. The proposed urinary target for AB-CHFUPYCA is the HO-cyclohexyl-metabolite. However, further studies should be performed to estimate degree of biliary elimination of the compounds and identify possible fecal analytical targets.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

UPLC-MS/MS analysis of illicit drugs in wastewater in the city of Lisbon and Almada between 2014 - 2016

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The European Drugs Report of 2016 states that the level of purity or potency of most illicit substances is high or increasing, as well as a slight increase in the estimated consumption of the most common drugs has been observed.

Monitoring drug abuse all over Europe is a major and ongoing project that involves national and European health and regulatory authorities. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has been supporting all the initiatives that may lead to a better and more precise knowledge of the drug abuse problem. The Sewage Analysis Core group Europe (SCORE) and the ES1307 COST action (Sewage Biomarkers Analysis for Community Health Assessment emerged as promising networks and wastewater (WW) analytical tools dealing with a different and quicker approach for obtaining estimates of drug abuse at national and European levels.

Two major Sewage Treatment Plants (STP) in the region of Lisbon (Lisbon-Alcântara and Almada-Mutela) have been regularly participating in the SCORE studies since 2014 contributing with qualitative and quantitative data on the major drugs and metabolites under study and some others in a total of 15 drug biomarkers.

The detection of drug residues (unaltered drug or drug's main metabolites) requires analytical techniques with high selectivity and specificity as the LC-MS/MS.

In this paper, an UPLC-MS/MS method is presented as developed for detection and quantification of illicit drugs in the wastewater influents of the two STPs in the region of Lisbon between 2014 and 2016 in the framework of the SCORE project. The UPLC-MS/MS method developed, and sample preparation procedures for the quantitative analysis of Morphine, Cotinine, 6MAM, MDA, MDMA, Amphetamine, Methamphetamine, Mephedrone, Ketamine, Benzoylecgonine, Cocaine, Cocaethylene, EDDP, Methadone and THC-COOH showed a recovery range from 47% to 105% and LOQ values in the range of 1 to 10 ng/mL. The prevalence of the substances in the WW samples analysed were: Morphine, Cotinine, Amphetamine, Methamphetamine, Benzoylecgonine, Cocaine, EDDP, Methadone and THC-COOH (100%); MDMA (95%); Cocaethylene (92%) 6MAM (86%), Ketamine (24%), Mephedrone (16%) and MDA (2,6%).

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The UPLC-MS/MS method developed showed adequate levels of performance to monitoring Illicit drugs in wastewater samples.

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Occurrence of Illicit Drugs in Aqueous Environment and Removal Efficiency of Wastewater Treatment Plants

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Abstract

Illicit drugs are considered as emerging contaminants of concern that have become an interesting issue for the scientific community from last few years due to their existence in the water environment. A number of the literature has revealed their occurrence in the environment. This is mainly due to the fact that some drugs are partially removed during wastewater treatment processes, and remaining being able to enter the environment and contaminate surface and groundwater and subsequently, drinking water. Therefore, this paper evaluates the occurrence of key illicit drugs in wastewater (influent and effluent) samples in 4 wastewater treatment plants across Adelaide, South Australia over a one year period. This paper also compares the efficiency of wastewater treatment plants adopting different secondary processes, such as activated sludge reactor, sequence batch reactor or membrane bioreactor in the removal of selected illicit drugs, especially in the context of which technology has higher removal rates.

The influent and effluent samples were analysed using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). The levels of drugs detected were in the range of mg/L – ng/L in effluent samples; thus emphasising the influence on water quality of receiving water bodies and the significance of removal efficiency of WWTPs. The results show that the drugs responded differently in the removal depending on the treatment processes used by the WWTPs. The wastewater treatment plant operational with activated sludge reactor followed by chlorination disinfectant process presents higher removal rates while sequence batch reactor was least efficient. Although the concentrations are not high in effluent water but cannot be ignored, as they may be 'pseudo persistent' or become persistent with prolonged population usage and environmental exposure.

***Keywords*—**Illicit drugs, removal efficiency, treatment technology, wastewater

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ES1307 *"Sewage biomarker analysis for community health assessment"*

A Novel Route for Determining Public Health: Analysis of Oxidative Stress Biomarkers in Wastewater

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The UN have estimated that by 2050, 2.5 billion people will be living in cities resulting in 66 % of the world's total population living in urban areas.[1] This unprecedented rise of urbanisation has caused increasing demands on water infrastructure and stresses upon public health. Significant increases in both communicable and non-communicable diseases have resulted in global health concerns, however measuring population-wide health is difficult. Current routes to assessing community-wide health are based upon resources that are often bias and time intensive, such as hospital and GP surgeries data, prescription rates or surveys.[2] One of the biggest challenges to conventional public health assessments is that there is no capacity for real-time monitoring. This results in there being a significant time lag between when a public health evaluation is being made to when results are actually established. There is therefore a high chance that when the results are determined, the health status of that community might have changed. Implementing effective public health interventions is consequently very challenging and monitoring their impact extremely difficult. The complexities of these current routes have resulted in a call to develop innovative analytical techniques for rapid detection for the monitoring of public health and potential epidemics.

Waste-water based epidemiology (WBE) is a well-established, innovative technique for the analysis of human excretion products in wastewater in near-real time.[3] This can give valuable information on the health of populations as well as the potential to measure a community's exposure to external stresses.[4] This can be done by observing urinary biomarkers of oxidative stress which are a key characteristic of many acute and chronic diseases.[5–7] Our current study has focused upon developing analytical methods for quantifying biomarkers of oxidative DNA damage and lipid peroxidation in wastewater. The biomarkers 8-OHdg, 8-NO_2Gua and HNE-MA have demonstrated limits of detection between 0.01-0.05 µg/L and preliminary studies have observed HNE-MA in wastewater in concentrations ranging from 4.5-13.5 ng/L. Target biomarkers were extracted from wastewater using solid phase extraction (SPE) techniques and analysis has been performed using ultra-high performance liquid chromatography coupled to a Xevo TQD triple quadruple mass spectrometer. This could offer a novel element to present epidemiological studies by giving valuable information on oxidative stress levels in populations in near-real time.

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Modelling Illicit Drug Fate in Sewers for Wastewater-Based Epidemiology

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Since the development of wastewater-based epidemiology more than 15 years ago, there have been many studies aiming at improving the reliability of estimations by addressing the challenges and uncertainties existing in methodological steps. This primarily includes chemical analysis, population estimates, sampling, and excretion ratios. However, despite reported evidences, potential in-sewer transformation of biomarkers hasn't gained enough attention.

Sewer network is not only a system of collection and conveyance of wastewater, but also a bioreactor for chemical and microbial transformations. Wastewater composition at the wastewater treatment plant influent is impacted by the design features and the operational conditions of the sewer system leading to alteration of organic matters during the sewer hydraulic residence time. Consequently, biomarkers, as trace organic chemicals, can be influenced by physico-chemical and biological processes (fate processes) in the sewer. Ignoring the fate of biomarkers in the sewer may lead to significant under- or over-estimation of consumption estimates in wastewater-based epidemiological studies.

Considering these challenges, reporting on chemical stability in terms of percentage removal efficiency or correction factors (lumped factors that include excretion ratio, in-sewer transformation etc.) may not be a reliable source of information for the estimation of substance load at the point of excretion. Therefore it is useful to develop models that can be used to predict in-sewer transformation of biomarkers, which eventually would lead to more accurate estimation of substance consumption.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Trends in methamphetamine and ketamine use in major Chinese cities from 2012 to 2016

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Methamphetamine (METH) and ketamine (KET) are two most abused drugs in mainland China. In the past several years, seizure of the two drugs in China has been ranking first and second, respectively, among illicit drugs. Water-based epidemiology is a promising approach to monitor drug abuse. This approach was first applied in four megacities in China in 2012. From 2014 to 2016, we performed annual nation-wide reconnaissance to examine use of the two drugs in major Chinese cities. In 2016, wastewater samples from up to 138 wastewater treatment plants (WWTPs) in 33 major cities were collected. No clear geographic trend existed in METH loads (normalized to 1000 inhabitants per day), whereas KET loads were much higher in cities in central and south China. METH loads decreased from 2014 to 2016 at 24 out of 28 WWTPs that were both sampled in 2014 and 2016, stayed at roughly same levels at 3 WWTPs, and increased at only 1 WWTPs. At the all the 17 WWTPs where KET loads were greater than 4 mg/1000 inh/d, KET loads all decreased dramatically (by 74.1% in average). Significant decrease in METH and KET loads in wastewater coincided with significant decrease in METH and KET seizure in 2014. In contrast, at the majority of the WWTPs that were both sampled in 2012 and 2014, no significant increase or decrease in METH and KET loads was observed. There results imply that METH and KET abuse in major Chinese cities (very likely in other Chinese cities too) have decreased significantly since 2014. This decrease may be attributed to the “Thunder Anti-Drug Movement” launched by drug police across China since 2013.

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ES1307 *“Sewage biomarker analysis for community health assessment”*

Estimation of the consumption of illicit drug uses in prisons and in the general population in France using wastewater analysis

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Drug use in prisons is currently recognized, but there is a lack of qualitative and quantitative data on this subject. Data exist, often linked to seizures and to the feedback of prison staff and medical personnel. But, there is a need for more objective view of these practices in order to enhance risk reduction and prevention policies. In this specific context of a closed sector, the estimation of drug consumption by the analysis of wastewater is well-adapted to obtain objective data by maintaining the anonymity of the prisoners and the staff.

A comparative study on three prisons has been carried out in France: several sampling campaigns have been conducted during one year in order to assess temporal and spatial variations in consumption of cocaine, cannabis, amphetamine, opiates and some new psychoactive substances (NPS). In parallel, measurement campaigns were managed in neighbouring urban wastewater treatment plants in order to assess consumption of the local population.

The practical achievement of the study did not encounter any particular problems from the time when it has been adapted to the conditions of detention.

Results demonstrate a real use of drugs inside prisons, quite similar from one to another, with temporal fluctuations. Significant variations in the quantities consumed are observed between the prison and the local population.

These findings provide data to better understand the problem of drug use in prison. Thus, this methodology can be used to adapt the prevention and harm reduction policies within the prisons.

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ES1307 *"Sewage biomarker analysis for community health assessment"*

Development of wide-field proteomics methods for water fingerprinting applied to public health

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Abstract max 350 words. Arial 11 – Justify.

Proteomic analysis of serum and urine is well established for identification of biomarkers of disease. Most notably for cancer markers such as increases in prostate specific antigen (PSA) but also for biomarkers of inflammation, such as C-reactive protein (CRP). These methods are man power-intensive in acquisition and analysis of samples, as a result of the need for individual samples from thousands of study participants. Wastewater-based epidemiology (WBE) is a growing field that aims to survey larger populations with more streamlined analyses.

WBE approach has been applied previously to study small molecules, determining the spatial and temporal differences in the use of drugs of abuse, for example. However, the approach being applied to identification of protein material is yet to be explored.

The following project aims to develop a non-biased and wide reaching method for proteomic analysis of wastewater directed towards community-wide public health concerns, such as spread of infection. Protein components are to be separated from other material present in wastewater, enzymatically digested and analysed using liquid chromatography in conjunction with high-resolution (Quadrupole-Time-of-Flight, QToF) mass spectrometry. Such a method provides unique advantages in that data is not biased, is able to identify a large array of species simultaneously and can be retrospectively re-analysed following future identification of new biomarkers. Early phases of the project will focus on identification and quantitation of clinically recognised proteins related to inflammation/infection responses and potentially progress to identification of species from a large database.

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Concentration and enantiomeric profiling of ketamine and norketamine in urine, wastewater and receiving water

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Enantiomeric analysis along with parent drug to metabolite ratio comparison in different matrices, from urine, to receiving wastewater and surface water, can be a useful tool to help assess the fate of the drug in question in the environment. Here, for the first time, we determined the enantiomeric composition and concentration ratio of ketamine and its major metabolite norketamine in urine, influent wastewater, effluent wastewater and receiving water with SPE-chiral-HPLC-MS/MS method.

EFs (enantiomeric fractions) of ketamine fluctuated around 0.5 in all studied matrices, showing its weakly stereoselective nature in the environment. By comparison, EFs of norketamine changed with the type of matrix. It was enriched with S(+)-norketamine in most urine samples, with the EFs ranging from 0.41 to 0.93. In influent wastewater, R(-)-norketamine became the enriched one, suggesting S(+)-isomer perferred degradation might occurred during the sewer. The variation of ketamine: norketamine ratio in urine and influent wastewater also supported the hypothesis of degradation, with the ratio in the former (0.45 ± 0.18) much lower than that in the latter matrix (5.48 ± 1.91). It differed from the results of previous studieds on nonketamine stability in the wastewater, which found it was quite stable. Things became a little bit different in effluent wastewater, where Norketamine presented as a nearly racemic mixture, indicating that S(+)-norketamine enrichment took place during the wastewater treatment. Much more comprehensive work need to be done to explore the reason why S(+)-norketamine enrichment occurred, whether it was resulting from ketamine degradation, or from the perferred R(-)-norketamine degradation. In receiving water samples collected from Shenzhen River, ketamine and norketamine were both nearly racemic. Such a result was of significant environmental relevance as most ecotoxicological studies were at racemate level.

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